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Effects of 5-HT2 receptor ligands on tail pinch-induced stress responding and open field behavior

John K. Hearn
Louisiana State University and Agricultural and Mechanical College

EFFECTS OF 5-HT_2 RECEPTOR LIGANDS ON TAIL PINCH-INDUCED STRESS RESPONDING AND OPEN FIELD BEHAVIOR

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John K. Hearn
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Abstract

Stress is known to exert an influence on neuroendocrine, autonomic, hormonal, and immune functioning. As a result of the debilitating effects of stress on numerous bodily systems, there exists a large body of research devoted to the etiology, physiological sequelae, and treatment of the condition. Further, the neurotransmitter serotonin (5-HT) has been implicated in stress responding. Presently, there is conflict in the literature as to the precise role serotonin plays in mediating the stress response. This study was an attempt to further elucidate the role of 5-HT in mediating an organism's response to tail pinch stress and the open field. Previously, we have demonstrated that peripheral administration of the broad-spectrum 5-HT_2 agonist, DOI, reduces stress responding in rats subjected to a tail pinch stressor (Hawkins, et al., 2002). This effect was fully blocked by peripheral coadministration of the broad spectrum 5-HT_2 antagonist, ketanserin. The present study examined further the role of the 5-HT_2 receptor subclass in mediating the stress response. We employed antagonists that selectively target either the 5-HT_{2A} or 5-HT_{2C} receptor in an effort to clarify the relative importance of each of these receptors in mediating the stress response. These compounds were injected subcutaneously in an effort to black the effects previously seen with DOI. DOI attenuated rearing and oral behavior directed at food, while increasing the frequency of head and body shakes in the open field. DOI-induced head shakes were blacked by the 5-HT_{2C} antagonist, SDZ SER 082, as well as by a combination of SDZ SER 082 and the 5-HT_{2A} antagonist, spiperone. Implications for the 5-HT_2 receptor subclass in mediating stress responding are discussed.

Introduction

Stress can be described as an organism's response to any influence exerted by environmental or endogenous factors that disrupt homeostatic mechanisms within the organism. These influences, or *stressors*, can be physical or psychological in nature, and have wide-ranging effects on neuroendocrine, autonomic, immune, and hormonal function. In humans, stress has been linked to a broad spectrum of illnesses, including major depressive disorders, Alzheimer's disease, general neurodegeneration, hypertension, and asthma (McEwen and Stellar, 1993). Given the well-known debilitating effects of stress, there exists a large body of literature aimed at elucidating the etiology, physiological sequelae, and treatment of the condition.

General Theories of Stress

In Walter Cannon's classic "fight-or-flight" paradigm, an organism's sympathetic nervous system is activated in response to physical and emotional stressors. In the case of a physical threat, the "fight or flight" reaction is a near-instantaneous sympathetic response that allows an organism to engage, or escape from a perceived environmental threat, such as a natural predator. Cannon noted that milder emotional states such as joy, grief, or disgust were capable of prompting sympathetic arousal as well (Cannon, 1929). Despite categorical distinctions, physical and emotional stressors were thought to prompt similar, if not equal activation of the sympathetic nervous system. Additionally, Cannon coined the term *homeostasis* in reference to an organism's ability to maintain internal constancy in the face of great changes in the surrounding environment.

Building upon the work of Cannon, Austrian physician-endocrinologist Hans Selye created a theoretical model aimed at describing an organism's nonspecific responses to

stress. Observing several common features of "sickness" in patients diagnosed with a wide range of illnesses, Selye developed a model termed the *General Adaptation Syndrome* (*GAS*), which outlined the physiological processes associated with the stress response (Selye, 1956). The *GAS* offered no theoretical distinctions between the body's response to emotional or physiological factors, be they positive or negative. Selye argued that the body responded in a *nonspecific* manner, via the *GAS*, to an essentially infinite amount of *specific* stressors (Selye, 1956). Perhaps most notably, Selye theorized that neuroendocrine responses to stress were intrinsically related to immune functioning. As such, many ailments resulted not from a specific influence, such as intoxication or infection, but from the inability of an organism to respond appropriately to the stressor through activation of the *GAS*. Thus, when an organism failed to meet the demands placed upon it by stress, certain physiological manifestations such as ulceration and hypertension would surface.

The theories of Cannon and Selye provide groundwork for understanding the physiological responses associated with stress, particularly with regard to nonspecific sympathetic arousal, which will be discussed later. In contrast, the recent work of James Deakin and Frederico Graeff has culminated in a theoretical model that distinguishes between various types of stressors, while proposing distinct neural mechanisms associated with each of them (Graeff, Guimares, Andrades, and Deakin, 1996). This particular theory hinges largely on an organism's response to *proximal* and *distal* threats (Graeff, et al., 1996). Potential (distal) threats are theorized to activate neural substrates that mediate anticipatory anxiety in situations where an organism must evaluate a possible threat. Conversely, proximal threats are thought to activate a neural mechanism

that generates intense panic in response to immediate threats such as physical pain, asphyxia, or impending attack by a predator (Graeff, et al., 1996). Although distinct serotonergic pathways are hypothesized to play a role in the response to each class of stressor, each mechanism plays a common role in activating sympathetic arousal, vigorous escape behavior, and analgesia (Graeff, 1993).

Physiological Effects of Stress

Sympathetic Nervous System. It is difficult to discuss stress responding without devoting significant attention to the sympathetic nervous system. Activation of the sympathetic nervous system is known to effect powerful short-term changes in the body, largely through the release of catecholamines. Epinephrine and norepinephrine are most commonly associated with this process which is thought to promote beneficial behavior in situations of "fight or flight" (Udelsman and Holbrook, 1994). A cursory review of the sympathetic effects on an organism reveals the utility of this system in the activation of vigorous behavior. Among the changes induced by this response are increases in arterial blood pressure, increased blood flow to active muscles, decreased blood flow to organs not aiding intense motor activity, increased cellular metabolism, increased glycolysis in muscles and the liver, as well as increased muscle tone (Guyton and Hall, 1996). The specific role of the catecholamines in mediating the stress response will be discussed later.

The Hypothalamic-Pituitary-Adrenal Axis. The HPA axis is a set of structures in the brain and peripheral nervous system regarded as an integral mediator of the stress response. Acting in concert with the sympathetic nervous system, the primary function of the HPA axis is to facilitate the rapid release of glucocorticoid hormones into the bloodstream during stress responding (Chrousos, 1995; Herman, Prewitt, and Cullinan,

1996). Much like the effects of catecholamines, glucocorticoids allow vigorous activity by promoting glucose release in the liver, increased cardiovascular activity, and deactivation of nonessential endocrine systems (Munck, Guyre, and Holbrook, 1984). In essence, the HPA axis primes the body for adaptive behavior that benefits short-term survival in situations that are acutely stressful or threatening. Additionally, the HPA axis seems to function in the long-term as a physiological coping mechanism, a role mediated by a feedback loop that will be described in greater detail later.

Stressors initiate stress responding at the level of the hypothalamus, the first component in the triad of structures that comprise the HPA axis. Here, corticotropin-releasing factor (CRF) is released from neurons originating in the paraventricular nuclei (Makara, Stark, Karteszi, Palkovits, and Rappay, 1981). CRF is then transported through the hypophyseal portal system, arriving at the anterior pituitary gland, where it stimulates the production of adrenocorticotropin, or ACTH (Hodges, 1984). ACTH is then secreted into the bloodstream, eventually arriving at the adrenal cortex where it stimulates increases in cyclic adenosine monophosphate (cAMP) levels and production of the glucocorticoid cortisol (corticosterone in rats) (Honn and Chavin, 1977). At this level of the HPA axis, cortisol is secreted into the bloodstream from the adrenal cortex.

Cortisol serves multiple functions in the central and peripheral nervous systems. Noted mainly for its anti-inflammatory properties, cortisol is also a powerful immunodepressant. Additionally, cortisol plays a role in maintaining water and electrolyte concentrations, gastric secretions, as well as promoting lipid, carbohydrate and protein metabolism (Simmons, Miles, Gerich, and Haymond, 1984). Cortisol also induces short-term increases in metabolism, promoting vigorous “fight or flight” behavior

as well as tissue repair in injured organisms, clearly adaptive effects with respect to physical stress (Udelsman and Holbrook, 1994; Kudsk and Mirtallo, 1983).

Negative Feedback in the HPA Axis. Chronically elevated glucocorticoid levels have been shown to adversely affect metabolic processes, disrupt the immune response, and play a role in the etiology of depression and other neurobiological disorders (Rebuffe-Scrive, Walsh, McEwen, and Rodin, 1992; Dhabhar and McEwen, 1997; Mizoguchi, Yuzurihara, Ishige, Sasaki, Chui, and Tabira, 2001). Thus, there is an apparent need for a regulatory mechanism that terminates stress responding, as continued elevation of glucocorticoid levels is maladaptive (McEwen, De Kloet, and Rostene, 1986). Cortisol plays an important role in regulating basal and stress-evoked levels of ACTH through the initiation of a negative feedback loop involving the hypothalamus, pituitary, hippocampus, amygdala, septum and other midbrain structures (McEwen, et al., 1986). Corticosteroids have been shown to have a *direct* inhibitory effect at the level of the hypothalamus and pituitary, resulting in decreased secretion of CRF and ACTH, respectively (Jacobson and Sapolsky, 1991). Other brain structures, most notably the hippocampus and frontal cortex have been shown to inhibit HPA axis activity by decreasing CRF and vasopressin secretion (Jacobson and Sapolsky, 1991). Conversely, certain nuclear groups of the amygdala have been shown to activate the HPA axis by stimulating increases in ACTH and cortisol release (Van de Kar & Blair, 1999).

Monoaminergic Regulation of Stress

The monoamines are found throughout central and peripheral tissues and are subdivided into two classes: the catecholamines and the indolamines. Both groups exert varying degrees of influence on stress responding.

Catecholamines: A Brief Overview. Norepinephrine and epinephrine, the catecholamines most relevant to the study of stress responding, are secreted peripherally by postganglionic sympathetic nerves and the adrenal medullae. In the central nervous system, the bulk of norepinephrine is produced in the locus coeruleus, although various nuclear groups of the hypothalamus, amygdala, and other groups synthesize the neurotransmitter as well (Pacak, Palkovits, Kopin, and Goldstein, 1995). Dopamine, a third catecholamine that plays a role in stress responding, is manufactured in the brain primarily by the substantia nigra and surrounding nuclear groups.

Catecholamines and Stress. Generally, the catecholamines are thought to stimulate activity in the HPA axis (Herman, et al., 1996). Exposure to a variety of physical and psychological stressors has been shown to elevate levels of norepinephrine in the hypothalamus, amygdala, and other brain structures intrinsically related to activation of the HPA axis (Pacak, et al., 1995). Introcerebroventricular (ICV) injections of norepinephrine and epinephrine have been shown to increase plasma ACTH levels and induce an increase in hypophyseal portal levels of CRF (Szafarczyk, Malaval, Laurent, Gibaud, and Assenmacher, 1987; Plotsky, 1987).

Lesion and stimulation studies have been integral to our understanding of catecholaminergic systems in the HPA axis, as well. Chemical lesion of the ventral noradrenergic bundle, a major adrenergic pathway innervating the hypothalamus, results in a drastic decrease in blood levels of CRF as measured in the hypophyseal portal system, as well as decreased hypothalamic levels of norepinephrine and epinephrine (Guillaume, et al., 1987). Stimulation of the same nerve bundle has been shown to increase plasma corticosterone levels in rats (Saphier and Feldman, 1989). Such findings

suggest a stimulatory role of the catecholamines in HPA axis functioning (Cunningham and Sawchenko, 1988; Cunningham, Bohn, and Sawchenko, 1990).

Serotonin and Stress

Since its discovery in the 1930s, serotonin (5-HT, 5-Hydroxytryptamine; see Figure 1) has become the focus of intense research in diverse fields including biochemistry, psychology, and neurophysiology. Initially detected in high concentrations in the gastrointestinal mucosa,

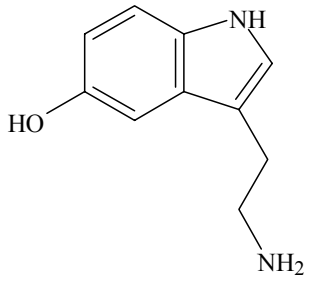

Figure 1: Serotonin

serotonin was later isolated in platelets, functioning as a vasoconstrictor upon its release into clotting blood (Erspamer, 1966; Rapport *et al*., 1948). Additionally, it was discovered that patients suffering from intestinal enterochromaffin tumor excreted relatively large volumes of the serotonin metabolite 5-hydroxyindoleactic acid in urine, a prominent marker that is still used in screening for gastrointestinal cancer today (Wilander, Lundqvist, and Oberg, 1989). This excess secretion of 5-HT, termed carcinoid syndrome, produced cutaneous flushing, diarrhea, bronchospasm, and right-sided valvular lesions, as well as psychotic effects similar to those produced by lysergic acid diethylamide (LSD). This behavioral link to LSD was one of the first pieces of data suggesting a presence of 5-HT in the central nervous system. In fact, serotonin *is* present throughout the CNS, where it

functions as a neurotransmitter, and in some cases, a neuromodulator, eliciting a wide range of acute and generalized actions in multiple bodily systems.

Serotonin Receptor Subtypes

In evolutionary terms, serotonin is one of the oldest biologically active substances, perhaps even predating the existence of adrenergic and cholinergic neurotransmitters (Venter, et al., 1988). In addition to humans and other mammals 5-HT is also present in mollusks, arthropods, insects, as well as some fruits and nuts (Feldman and Lee, 1985; Tarchalska-Krynska and Kostowski, 1976). Interestingly, the structural features of the serotonin molecule have been conserved over millions of years, while 5-HT receptors themselves have evolved into pharmacologically distinct networks (Venter, et al., 1988).

Today, a concise system of categorizing 5-HT receptor subtypes has been developed. There are currently seven families of 5-HT receptors, numbered 5-$HT_{1\text{-}7}$. Further subdivisions exist within these families (e.g., 5-$HT_{1A,1B,1C}$) Of the families mentioned above, 5-HT_1, 5-HT_2, and 5-HT_3 receptors have been researched in greatest depth, and will be discussed in relation to their relevance to the study of stress and anxiety.

The 5-HT_1 Receptor Subtype: An Overview. 5-HT_1 receptors are found throughout the central and peripheral nervous system, where they share a common role in decreasing serotonergic activity (Marcinkiewicz, Verge, Gozlan, Pichat, and Hamon, 1984; Radja, Laporte, Daval, Verge, Gozlan, and Hamon, 1991; Hoyer, et al., 1994). The 5-$HT_{1A, 1B,}$ and $_{1D}$ receptors have been studied in greatest detail, and appear to function both presynaptically and postsynaptically. In the former case, 5-HT_1 receptors serve as autoreceptors, reducing synthesis and firing rates of 5-HT neurons (de Montigny and Blier, 1992; Middlemiss, Bremer, and Smith, 1988; Graeff, 1993). Postsynaptic 5-HT_1

receptors are found in various forebrain sites, especially the hippocampus, where they are thought to have a hyperpolarizing effect, thereby reducing the effects of 5-HT in the synapse. The 5-HT_{1C} receptor subclass has been found to share sufficient features with the 5-HT_2 family that it has been renamed $_{2C}$, accordingly (Humphrey, Hartig, and Hoyer, 1993). Little is known about the 5-HT_{1E} and $_{1F}$ families, though they share many of the primary features of the 5-HT_1 family. Development of specialized ligands for these receptors will facillitate subsequent pharmacological categorization.

Clinical Significance of the 5-HT_1 Receptor Family. Selective and/or partial agonists of the 5-HT_{1A} receptor have proven efficacious in the treatment of depression and anxiety, suggesting that both disorders have a common root involving serotonergic function (Deakin, 1998). Buspirone (Buspar), the first clinically available drug of this nature, is a selective partial agonist at the 5-HT_{1A} receptor that has garnered attention as a non-sedative anxiolytic, effective in the treatment of generalized anxiety disorder (Gammans, Stringfellow, Hvizdos, Seidehammel, Cohn, Wilcox, et al., 1992). Notably, the anxiolytic and antidepressant effects of buspirone and other 5-HT_{1A} agonists are generally only observed after one to three weeks of chronic administration. Such findings suggest that a secondary mechanism, such as presynaptic autoreceptor downregulation may be responsible for the therapeutic benefit seen after treatment with 5-HT_{1A} agonists.

Behavioral Effects of 5-HT_1 Receptor Ligands. Animal studies utilizing 5-HT_1 ligands have produced findings that vary across experimental models. As mentioned earlier, the delayed therapeutic effect of buspirone in humans seems to suggest that an increase in 5-HT activity secondary to downregulation of presynaptic 5-HT_1 receptors is ultimately responsible for reducing anxiety. In rats, however, *acute* central and peripheral

injections of the 5-HT_{1A} agonist 8-OH-DPAT have been shown to attenuate stress responding in the elevated T-maze, various restraint paradigms and the elevated plus maze (Graeff, et al., 1996; McBlane and Handley, 1994; Kennett, Dourish, and Curzon, 1987). Similarly, acute systemic administration of the 5-HT_{1A} agonist buspirone has been shown to reduce ultrasonic vocalization, while releasing suppressed locomotor activity in response to foot shock in rats, both anxiolytic effects (Rowan, Cullen, and Moulton, 1990). Upon initial examination, these findings seem compatible with findings that 5-HT_1 agonists are anxiolytic in humans. However, the delayed therapeutic effects seen in humans suggest that a different mechanism, such as presynaptic autoreceptor downregulation or postsynaptic upregulation, may be at work.

5-HT_1 Receptors and Open-field Behavior. The open field is an experimental environment that assesses animal behavior on a range of variables that include locomotion, rearing, grooming, and body posture. Increases in locomotion and rearing are classified as exploratory behavior and are commonly associated with anxiolysis. Increases in grooming, or the presence of a creeping gait and elongated crawling posture near the periphery of the field are regarded as anxiogenic behaviors. Head and body shakes are also observed in the open field, but are typically regarded as artifacts of drug administration rather than an indicator of a specific anxiolytic or anxiogenic effect.

The administration of a 5-HT_{1A} agonist in rats has consistently been shown to alter locomotion in the open field. Subcutaneous administration of the 5-HT_{1A} agonists 8-OH-DPAT, ipsapirone, and buspirone all reduced locomotion and rearing in the open-field, both anxiogenic effects (Frances, Khidichian, and Monier, 1990, Kennett, et al., 1987). Similarly, injections of the 5-HT_{1A} agonists flesinoxan and 8-OH-DPAT both reduced

instances of rearing, while *increasing* movement along the periphery of the open field, effects deemed by the authors to be anxiogenic (Ahlenious, Larsson, and Wijkstrom, 1991). Though these studies suggest that administration of a 5-HT_{1A} agonist results in increased anxiety in the open field, contrary findings have been reported. Intra-hippocampal injections of the 5-HT_{1A} agonist buspirone have been shown to *increase* exploratory behavior in the open field, as well as the elevated plus-maze (Kostowski, Plaznik, and Stefanski, 1989).

5-HT_{1A} Receptors and the HPA Axis. There is limited evidence to support an interaction between HPA axis functioning and the 5-HT_{1A} receptor. In low doses, microinjections of the 5-HT_1 agonist 8OH-DPAT into the PVN or third ventricles resulted in decreased plasma levels of corticosterone, whereas high doses produced the opposite effect (Welch, Farrar, Dunn, and Saphier, 1993). The latter finding has been replicated, with 5-HT_1 agonists consistently reported to increase CRF release from the hypothalamus, followed by rises in serum levels of ACTH and corticosterone (Feldman, Newman, Weidenfeld, 2000; Hemrick-Luecke and Evans, 2002).

Genetic Alteration of the 5-HT_{1A} Receptor. Recent genetic manipulation of the 5-HT_1 receptor level has provided compelling evidence for a role of 5-HT_{1A} receptors in stress and anxiety. Three strains of knockout (KO) rats, altered to lack the 5-HT_{1A} receptor, have been shown to display increased anxiety in the open field, foot shock test, and a range of behavioral conflict paradigms (Gross, Santarelli, Brunner, Zhuang, and Hen, 2000). It should be noted, however, that differences in stress responding in 5-HT_{1A} KO rats seem to depend on the intensity of the stressor in certain strains of animals (Olivier, Pattij, Wood, Oosting, Sarnyai, and Toth, 2001). That is, certain strains of 5-

HT_{1A} KO rats do not exhibit higher baseline anxiety, but display enhanced responses to stressful stimuli, such as cage changes and induced hyperthermia (Olivier, et al., 2001).

The 5-HT_2 Receptor Subtype: An Overview. The 5-HT_2 receptor subclass is regarded traditionally as a mediator of certain peripheral actions of 5-HT, which include bronchoconstriction, platelet aggregation, and muscle contraction (Hoyer, et al., 1994). Centrally, 5-HT_2 receptors are likely involved in the mediation of anxiety, depression, and schizophrenia. Currently, three subtypes of 5-HT_2 receptors are recognized ($_{2A}$, $_{2B}$, and $_{2C}$)

Clinical Significance of the 5-HT_2 Receptor Family. Several ligands of the 5-HT_2 receptor have proven effective in the treatment of clinical disorders such as depression and schizophrenia. The 5-HT_{2A} receptor may play a role in the etiology of schizophrenia, based on the success of the antipsychotic drug clozapine, a potent 5-HT_{2A} antagonist (Huttunen, 1995). Clozapine, also a dopamine D_2 receptor antagonist, was an integral factor in the development of the serotonin-dopamine antagonist model of treatment for schizophrenia. This model called for the use of single compounds that were both selective antagonists at the 5-HT_{2A} receptor, as well as less potent blockers of the D_2 receptor (Huttunen, 1995). Risperidone, the first clinically available compound in this evolving class of antipsychotics, has shown promise in reducing the positive and negative symptoms of schizophrenia, without inducing the extrapyramidal symptoms characteristic of traditional anti-dopaminergics (Huttunen, 1995).

5-HT_2 antagonists have also shown promise recently in the treatment of clinical depression. Nefazadone, a 5-HT_2 selective antagonist, improved depressive symptoms with therapeutic benefits similar to those seen with serotonin-selective reuptake inhibitors

(SSRIs) and imiprimine, without inducing many of the adverse effects commonly seen in other classes of antidepressants (Davis, Whittington, and Bryson, 1997). Mianserin, a 5-$HT_{2A/2C}$ antagonist, has also proven effective in preventing sexual dysfunction associated with SSRIs when used in conjunction with the existing treatment regimen (Aizenberg, Gur, Zemishlany, Granek, Jeczmien, and Weizman, 1997). Similarly, mianserin positively augmented the symptoms of treatment-resistant depression when used in concert with the SSRI fluoxetine (Maes, Libbrecht, van Hunsel, Campens, and Meltzer, 1999).

Behavioral Effects of 5-HT_2 Receptor Ligands. 5-HT_2 agonists and antagonists have been tested extensively in several animal models of anxiety and stress. The findings are frequently inconsistent, suggesting complex interactions between various brain sites and 5-HT receptor subtypes in the mediation of stress and anxiety.

There are several studies that suggest decreased serotonergic activity at the 5-HT_2 receptor may be associated with decreased anxiety and stress responding. Many of these studies involve the administration of a selective antagonist. Intraperitoneal injections of SB242084, a selective brain-penetrating antagonist at the 5-HT_{2C} receptor, has been shown to increase social interaction in rats (Kennett, et al., 1997). Peripheral administration of mianserin, a non-selective 5-HT_2 antagonist, and SB 206553, a selective $_{2B/2C}$ antagonist, increased punished responding in the Vogel drinking test and increased open-arm entries in the elevated plus-maze, both anxiolytic effects (Griebel, Perrault, Sanger, 1997). In the rat ultrasonic vocalization test, social cohesion test, and the elevated T-maze, peripherally administered 5-HT_{2A} antagonists consistently produced

anxiolytic effects as well (Schreiber, Melon, and De Vry, 1998; Rademacher, Anderson, and Steinpreis, 2002).

Certain studies have found that increased serotonergic activity at the level of the 5-HT_2 receptor class results in anxiolysis. Intra-amygdala injections of the 5-HT_{2B} agonist BW723C86 increased social interaction in rats, an effect antagonized by oral pretreatment with the 5-$HT_{2B/2C}$ antagonist SB 200646A (Duxon, Kennett, Lightowler, Blackburn, and Fone, 1997). DOI, a nonselective agonist of the 5-HT_2 receptor, was found to reduce tail-pinch-induced stress responding when injected subcutaneously, or by a combination of subcutaneous and intracerebroventricular administration (Hawkins, Uzelac, Baumeister, Hearn, Broussard, and Guillot, 2002).

In the open field, limited findings suggest that increased serotonergic activity at the 5-HT_2 receptor correlates with a decrease in anxiety. DOI, a nonselective 5-HT_2 agonist, increased exploratory behavior in the open field, an effect which was fully antagonized by the 5-$HT_{2A/2C}$ antagonist ritanserin (Kaur and Ahlenius, 1997). Similarly, microinjections of nefazadone, a nonselective 5-HT_2 antagonist, into the basolateral amygdala, potentiated the anxiogenic effects of inferior colliculus stimulation in the open field (Maisonnette, Villela, Carotti, and Landeira-Fernandez, 2000).

5-HT_2 Receptors and the HPA Axis. DOI, m-CPP, Ro 60-0175 and quipazine have been shown to increase serum concentrations of corticosterone in rats (Hemrick-Luecke and Evans, 2002; Raghavendra and Kulkarni, 2000;). This effect can be abolished by co-administration of the highly selective 5-HT_{2A} antagonist M100907, but is only marginally affected by the selective 5-HT_{2C} antagonist SB 242084, suggesting a

prominent role of the $_{2A}$ receptor in activating the HPA axis (Hemrick-Luecke and Evans, 2002).

Genetic alteration of the 5-HT_2 receptor. Limited studies involving genetic manipulation of 5-HT_2 receptors have provided support for serotonergic involvement in stress responding, particularly with regard to the HPA axis. A recent finding has shown that deletion of the 5-HT_{2C} receptor leads to increased plasma concentrations of ACTH and corticosterone in rats subjected to repeated cage changes, suggesting hyperresponsiveness of the HPA axis in these animals (Chou-Green, Holscher, Dallman, and Akana, 2003).

The 5-HT_3 Receptor Subtype: An Overview. The 5-HT_3 receptor is the only member of the 5-HT family that operates as a ligand-operated ion channel, directly influencing potentials via stimulation of cation-selective channels (Derkach, Suprenant, and North, 1989; Malone, Peters, and Lambert, 1991). 5-HT_3 receptors are found at multiple locations within the peripheral and central nervous systems. Locations relevant to the study of stress and anxiety will be discussed subsequently.

Clinical Significance of the 5-HT_3 Receptor Family. The 5-HT_3 receptor is well known for its role in the mediation of the emetic response. A family of selective 5-HT_3 antagonists that includes granesitron, ondansetron, tropisetron, and dolasetron has proven highly effective in the treatment of emetic disorders. It has been suggested that these drugs operate by central antagonism of 5-HT_3 receptors in the chemoreceptor trigger-zone of the area postrema (Higgins, Kilpatrick, Bunce, Jones, and Tyers, 1989), though a peripheral mechanism mediated in the upper gastrointestinal tract through vagal afferents is likely also involved (Andrews, Davis, Bingham, Davidson, Hawthorn, and Maskell,

1990). Recent research suggests that the 5-HT_3 family of receptors is also involved in the etiology of a diverse array of clinical disorders that include anxiety, depression, psychosis, chronic pain, as well as drug and alcohol abuse (Greenshaw and Silverstone, 1997).

5-HT_3 Ligands and Stress. Compounds that selectively target the 5-HT_3 receptor have been tested extensively in various animal models of physical and psychological stress. Peripheral administration of selective 5-HT_3 antagonists has been shown to reduce stress responding, in terms of both physiology and behavior, suggesting an anxiolytic effect as a result of reduced serotonergic activity at the 5-HT_3 receptor. In rats, intraperitoneal injection of the 5-HT_3 antagonists ICS 205-930, GR 28032F, and ondansetron decreased stress-induced prolactin release, an anxiolytic effect (Jorgensen, Knigge, and Warberg, 1992, Nonaka, 1999). Ondansetron has also been shown to increase exploratory behavior in the open field, as well as negating the anti-exploratory effects of caerulein, a decapeptide, in the elevated plus-maze (Stefanski, Palejko, Kostowski, and Plaznik, 1992; Vasar, Peuranen, Oopik, Harro, and Mannisto, 1993).

There is a growing body of evidence that 5-HT_3 antagonists may exert their anxiolytic effects centrally. Injections of the 5-HT_3 antagonists tropesitron or ondansetron into the hippocampus and nucleus accumbens produced anxiolytic-like behavioral effects in the Vogel conflict test and the open field (Stefanski, Palejko, Bidzinski, Kostowski, and Plaznik, 1993). These antagonists also suppressed stress responding induced by electroconvulsive shock (Kostowski, Plaznik, Nazar, and Jessa, 1995).

Theoretical Bases for Current Research

5-HT_1, $5HT_2$ and perhaps 5-HT_3 receptors have been implicated in stress responding. Previously, we have demonstrated that subcutaneous injection of the 5-$HT_{2A/2C}$ agonist, DOI, dose-dependently reduces tail pinch-induced stress responding in rats (Hawkins, et al., 2002). Additional unpublished data from our lab show that this effect can be reversed in a dose-dependent fashion by subcutaneous co-administration of the 5-$HT_{2A/2C}$ antagonist Ketanserin. Further, we have observed that stress responding is not affected when 5-HT or DOI are injected into the cerebral ventricles, dorsal periaquaductal grey, or the amygdala. Therefore, the anxiolytic action of DOI appears to be mediated by a peripheral mechanism despite the fact that DOI and ketanserin cross the blood-brain barrier.

Other findings support the theory that increased serotonergic activity at the 5-HT_2 receptor results in decreased anxiety. Intra-amygdala microinjections of the 5-HT_{2B} agonist BW723C86 have been shown to increase social interaction in the rat (Duxon, et al., 1997). Similarly, intraperitoneal injections of the 5-HT_2 agonists TFMPP and mCPP have been shown to impair inhibitory avoidance in the elevated T-maze, an anxiolytic effect (Mora, et al., 1997). In the open field, DOI has been shown to increase exploratory behavior, an effect fully antagonized by the 5-$HT_{2A/2C}$ antagonist ritanserin (Kaur and Ahlenius, 1997).

In addition to the aforementioned pharmacological studies, genetic manipulation of the 5-HT_{2C} receptor has provided further support for the role of this receptor as an integral mediator of negative feedback in the HPA axis (Chou-Green, et al., 2003).

The current study investigated further the role of the 5-HT_2 receptor in the mediation of

stress responding in the rat. Experiments employed selective 5-HT_{2A} and 5-HT_{2C} antagonists in an effort to block the stress-related effects of peripherally administered DOI. Such experiments were intended to clarify the relative importance of these two receptor subtypes in mediating the behavioral effects of DOI.

Hypotheses

1. The non-selective 5-HT_2 agonist, DOI, injected subcutaneously, will decrease tail pinch-induced stress responding in rats.
2. The effect of DOI on tail pinch-induced stress responding will be reduced by peripheral co-administration of the selective 5-HT_{2A} antagonist, spiperone.
3. The effect of DOI on tail pinch-induced stress responding will be reduced by peripheral co-administration of the selective 5-HT_{2C} antagonist, SDZ SER 082.
4. Spiperone and SDZ SER 082 will have no effect on stress responding when administered alone.
5. Simultaneous administration of spiperone and SDZ SER 082 will result in greater antagonism of DOI's effects than that of either antagonist administered alone.
6. Subcutaneous injection of DOI will have an anxiolytic effect on animal behavior in the open field.
7. The effect of DOI on open field behavior will be reduced by spiperone, SDZ SER 082, or a combination of the two antagonists.
8. The administration of DOI, spiperone, SDZ SER 082 and combinations thereof, will not impair general motor function, as measured by rotarod performance and ambulation in the open field.

Drugs

Determination of Doses. In the case of DOI, drug was administered at doses that were based on previous work conducted in our lab. We have seen an effect of medium and high doses (0.5 and 1.0 mg/kg) of DOI on stress responding, with no effect following low dose injections (0.1 mg/kg) (Hawkins, et al., 2002). Doses of spiperone and SDZ SER 082 were determined based on an extensive search of the literature (see Tables 1 and 2). Highest priority was assigned to literature relevant to the study of stress and anxiety, particularly studies that employed subcutaneous administration of spiperone or SDZ SER 082. Studies utilizing alternate routes of systemic administration, such as interperitoneal injections, were considered, as well. Lastly, low priority was assigned to literature unrelated to stress, but that employed relevant compounds, a systemic route of administration and behavioral dependent variables.

5-HT$_{2A/2C}$ Agonist. DOI [(+/-)-1-(2,5-dimethoxy-4-iodophenyl)-2 aminopropane; Figure 2] is a potent serotonergic agonist with high affinity for the $_{2A}$ and $_{2C}$ receptors.

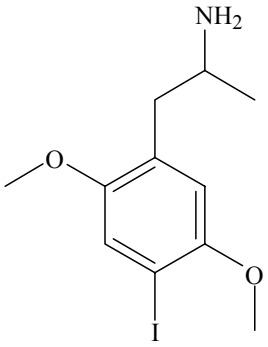

Figure 2: DOI

This compound has been tested in several models of anxiety and stress mentioned previously and is known to readily cross the blood-brain-barrier (BBB). DOI HCl was purchased from Sigma-Aldrich Corp. (St. Louis, MO).

5-HT_{2A} Antagonist. Spiperone (8-[3-(p-fluorobenzoyl)propyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one; Figure 3; Table 1) is classified as an antipsychotic with high affinity for 5-HT_{2A} receptors. It is also a selective D_2 antagonist and a weak antagonist of the 5-HT_{1A} receptor. It has been tested sparingly in animal models of anxiety and stress.

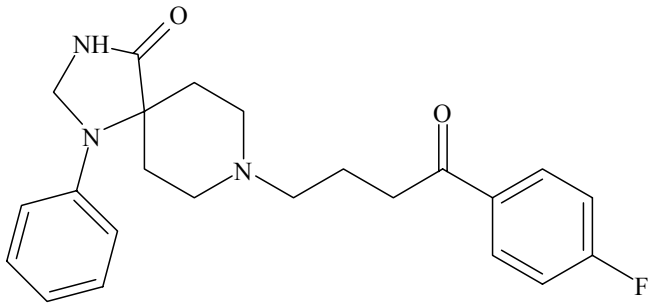

Figure 3: Spiperone

Due to the low solubility of spiperone in saline, it frequently has been administered as a suspension (e.g., Fujikawa, 1993, 1995; Kurashima, 1994, 1995; Matsumoto, 1988, 1989). The same method was employed in the current study. Spiperone HCl was purchased from Tocris Cookson Inc. (Ellisville, MO).

Table 1: References used to determine dose range for spiperone

Article Author	Dependant Variable	Dose Range	Route
Berendsen, et al., (1989)	Lip retraction	0.04-0.4 mg/kg	SC
Done & Sharp, (1992)	Hippocampal NE release	0.2-1.0 mg/kg	SC
Fregly & Rowland,(1988)	Isoproterenol consumption	0.1 mg/kg	SC
Fujikawa, et al., (1995, 1996)	Yawning	0.5 mg/kg	SC
Gartside & Cowen (1990)	ACTH release	1.0 mg/kg	IP
Inoue, et al., (1996)	Conditioned freezing	0.1-1.0 mg/kg	SC
Kennett, et al., (1987)	Restraint stress	0.1 mg/kg	SC
Kimura, et al., (1991)	Yawning	0.5 mg/kg	SC
Kostrzewa & Kastin, (1993)	Catalepsy	0.3 mg/kg	SC
Kurashima, et al., (1994)	Yawning, Oral Stereotopy, Body Temperature	0.5 mg/kg	SC
Kurashima, et al., (1995)	Prolactin secretion	0.5 mg/kg	SC

Table cont.

Matsumoto, et al., (1988; 1989)	Yawning, prolactin secretion	0.5 mg/kg	IP/SC
Ossowska, et al., (1982)	Catalepsy	0.2 mg/kg	SC
Rittenhouse, et al., (1994)	ACTH secretion	0.01-0.1 mg/kg	SC
Rittenhouse, et al., (1991)	Renin secretion	0.01-0.1 mg/kg	SC
Schreiber, et al., (1993)	Ethanol preference	0.05 mg/kg	SC
Sobrian, et al., (2003)	Grooming, oral stereotopy, locomotion	0.06-1.0 mg/kg	SC
Yamada, et al., (1986)	Post-decapitation convulsions	2.0 mg/kg	SC

5-HT_{2C} Antagonist. SDZ SER 082 ((+)-*cis*-4,5,7a,8,9,10,11,11a-Octahydro-7*H*-10-methylindolo[1,7-bc][2,6]-naphthyridine; Figure 4; Table 2) is a highly selective compound at the 5-$HT_{2B/2C}$ receptor with a very low affinity for 5-HT_{1A} receptors. SDZ SER 082 has been used extensively in an effort to differentiate the behavioral effects mediated by the 5-HT_{2A} and 5-HT_{2C} receptors. Notably, it has proven effective in blocking behavior induced by serotonergic agonists at the 5-HT_2 receptor, in various models of stress, as well as behavioral research unrelated to stress. SDZ SER 082 fumarate was purchased from Tocris Cookson Inc (Ellisville, MO).

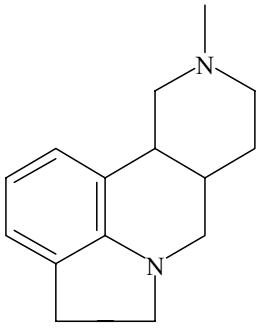

Figure 4: SDZ SER 082

Table 2: References used to determine dose range for SDZ SER 082

Article Author	Dependent Variable	Dose Range	Route
Goudie, et al., (1998)	Drug discrimination (clozapine)	0.25-1.0 mg/kg	SC

Table cont.

Koskinen, et al., (2000)	Five-choice serial reaction time task	0.1-1.0 mg/kg	SC
Krebs-Thompson, et al., (1998)	Locomotor activity	0.5-1.0 mg/kg	SC
Mora, et al., (1997)	Anxiety (elevated T-maze)	0.1-1.0 mg/kg	SC
Ouagazzal, et al., (2001)	Prepulse inhibition	1.0 mg/kg	SC
Varty, et al., (1999)	Prepulse inhibition	1.0 mg/kg	SC
Willins & Meltzer, (1997)	DOI-induced head twitches	0.3 mg/kg	SC

Method

The current study was composed of five experiments, each of which followed the same general format. Procedures common to all of the experiments will be described collectively, followed by specific methodological alterations unique to each experiment.

All drugs were stored at room temperature in their original containers until needed. Drugs were weighed at the start of each testing day and were diluted in sterile physiological saline (0.9%) to the desired concentration.

Sixteen male Sprague-Dawley rats between the ages of 8 and 12 weeks were used for each experiment. Animals were obtained from the Division of Laboratory Animal Medicine of Louisiana State University. Animals were housed individually with water and laboratory chow available ad-lib. The room in which the animals were housed were held at a constant temperature of 22 degrees C with lighting set on a 12-hour light/dark cycle, beginning at 07:00 hr.

Each experiment employed a within-groups design, consisting of three drug conditions and a control condition in which animals received saline vehicle. Order of presentation of experimental conditions was manipulated employing a modified Latin square design. Testing occured at three day intervals in an effort to maximize recovery time in the event that tail damage occurred during observation.

Experiment One: Peripheral DOI (5-$HT_{2A/2C}$ Agonist)

Animals were randomly assigned to one of four experimental conditions: DOI (0.1 mg/kg, 0.5 mg/kg and 1.0 mg/kg) and a saline control.

Experiment Two: Peripheral Spiperone (5-HT_{2A} Antagonist)

Animals were randomly assigned to one of four experimental conditions: Spiperone (0.1 mg/kg, 0.5 mg/kg, and 1.0 mg/kg) and a saline control.

Experiment Three: Peripheral SDZ SER 082 (5-HT2C Antagonist)

Animals were randomly assigned to one of four experimental conditions: SDZ SER 082 (0.1 mg/kg, 0.5 mg/kg, and 1.0 mg/kg) and a saline control.

Experiment Four: DOI + Single Antagonist

Animals were randomly assigned to one of four experimental conditions: DOI alone, DOI + Spiperone, DOI + SDZ SER 082, and a saline control.

Determination of Doses. Doses of DOI, spiperone and SDZ SER 082 were selected based on the outcome of Experiments 1, 2 and 3. In the case of DOI, the lowest overall effective dose in Experiment 1 was administered. The highest dose of each antagonist was used, provided that no dose of either drug was found to significantly alter stress responding when administered alone. If an antagonist was found to alter stress responding when administered alone, the highest *ineffective* dose was used in Experiment 4.

Experiment Five: DOI + Multiple Antagonists.

Doses of DOI, spiperone, and SDZ SER 082 were determined based on the outcome of experiments 1, 2 and 3. Animals were assigned to one of four experimental conditions: DOI alone, spiperone + SDZ SER 082, DOI + spiperone + SDZ SER 082, and a saline control.

Determination of Doses. As in Experiment 4, the lowest overall effective dose of DOI was administered (based on the results of Experiment 1). Similarly, the highest ineffective doses of both antagonists were co-injected with DOI in Experiment 5.

General Procedure

Rater Training Criterion. Data collectors were trained to 85% accuracy in both tail pinch and open field observations. Animals used for training purposes were not included as subjects in subsequent experiments. Data collectors learned to properly handle the animals, apply stressors, and record behavioral data. Training occurred in the presence of an experienced (previously trained) rater, who also observed and recorded behavioral data. Data collectors were required to successfully record each behavior to a degree of 85% concordance with the experienced rater in two consecutive 4-minute trials. These criteria applied to both tail pinch and open field observations.

Single Drug Injection Procedure. Prior to receiving an injection, animals were taken from their home cages, weighed, and their tails marked for tail pinch (as described in greater detail later). Animals were restrained by hand and received a single subcutaneous injection of drug or saline vehicle at the base of the neck. The total volume of drug injected was based upon the individual animal's weight and was administered in a volume of 1.0 ml per kg. After receiving injections, animals were returned to their home cages for a period of thirty minutes. At the conclusion of this waiting period, animals were placed in the tail pinch cage and exposed to the stressor described below.

Multiple Drug Injection Procedure. Combination agonist/antagonist injections were administered in a volume of saline calculated based on the individual animal's weight (1.0 ml per kg). The concentration of each drug was increased so that the total volume of injected solution was equal to that of single-injection animals. After receiving an injection, animals were returned to their home cages for a thirty-minute waiting period, prior to testing.

Testing

All experimental animals were observed under two conditions: the tail pinch stressor and the open field. Observation in the tail pinch cage always preceded the open field.

The Tail Pinch Stressor. Prior to injection, animals' tails were marked with a felt-tipped marker at a diameter of 4.3 mm. These measurements were made using standard metal calipers. The tails were remeasured and remarked prior to each tail pinch session to control for possible differences in diameter due to edema or growth of the animals.

The tail pinch cages were cleaned and dried before each trial. Animals were placed in the center of the suspended wire cage, the length of the tail was guided through the cage's floor, and a clamp was applied at the previously marked diameter. The clamp was fashioned from hemostatic forceps, padded with plastic tubing to avoid tail damage. Animals were observed for a period of four minutes. After observation, the forceps were removed and the animals were returned immediately to their home cages.

Dependent variables in the tail pinch. Animals' behavior was assessed on the following five variables during exposure to the tail pinch stressor:

1. Oral behavior directed at food was defined as the total amount of time an animal licked, gnawed, or chewed laboratory chow.
2. Oral behavior not directed at food was defined as the amount time an animal licked or bit the cage, or engaged in oral behavior without having food in its mouth (e.g., chattering of the teeth).

3. Grooming was defined as the amount of time an animal engaged in licking its own body, fluffing or wiping its facial hair or whiskers with the forepaws, or directing any oral behavior toward its tail.
4. Vocalizations were defined as the number of vocal emissions over the course of the testing period.
5. Fecal boli were recorded as the number of boli produced over the course of the testing period.

After completion of the tail pinch, two measures were made of the animals' oral behavior directed toward lab chow during the testing period:

6. Eating was defined as the amount of lab chow an animal ingested over the course of the testing period. This measure was calculated by subtracting the post-test weight of the lab chow from the pre-test weight.
7. Gnawing was defined as the amount of lab chow that was shredded, but not ingested by the animal over the course of the testing period. This measure was made by weighing the shredded chow that fell through the grating of the cage during testing.

Open Field Testing. Forty-five minutes after injection (roughly ten minutes after the conclusion of the tail pinch), animals were removed from their home cages and placed in the open field for observation. The open field is an open-topped, Plexiglas box measuring 2'x2'x2'. The floor of the apparatus is divided into four quadrants, each a 12" square. A 75-watt light bulb is positioned directly above so that it shines down into the open field. All animals were placed in the same predetermined quadrant and

were observed for a period of four minutes. Following open field testing, animals were returned to their home cages.

Dependent Variables in the Open Field. Behavioral observation in the open field involved measurement of the following five variables:

1. Line crosses were defined as the number of times an animal passed completely (all paws) into a different quadrant of the open field.
2. Rearing was defined as any instance in which an animal lifted both front paws simultaneously from the floor of the open field.
3. Head shakes were defined as any instance in which an animal vigorously shook its head from side to side.
4. Wet dog shakes were defined as any instance in which an animal shook its head in combination with the upper torso.
5. Freezing was defined as the total amount of time an animal was completely motionless, with the exception of breathing.

Rotarod. Twenty-four hours after the conclusion of the final day of tail pinch and open field testing, each animal was trained on the rotarod apparatus. The rotarod consists of a textured steel drum (7.2 cm in diameter) that rotates at adjustable speeds set by the experimenter. During training and testing, the rotarod was set to rotate at a velocity of 10 RPM. Animals received repeated trials on the rotarod until the training criterion of thirty seconds (without falling off) was met. Animals unable to reach the training criterion in twelve successive trials were excluded from subsequent testing.

Rotarod testing occurred twenty-four hours after training. Animals meeting the training criterion were administered drug or saline based on the experimental condition

assigned to them on the first day of stress testing. Injections were administered as previously described and animals were returned to their home cages. Thirty minutes later, animals were taken from their home cages and placed upon the rotarod apparatus. Animals were allowed to continue walking on the rotarod until thirty seconds passed, or the animal fell off, whichever occurred first.

Operational Definition of Anxiolytic and Anxiogenic Effects. Anxiety was inferred when behavior occurred as a result of the imposition of a stressor upon an animal. Consistent with convention in the existing literature (e.g. Adamec, Bartoszyk, and Burton, 2004; Adamec, Creamer, Bartoszyc, and Burton, 2004; Heilig, 2004; Sanchez, Gruca, Bien, and Papp, 2003; Haller and Bakos, 2002; Maslova, Bulygina, and Markel, 2002; Clark, et al., 2002; Radulovic, Ruhmann, Liepold, and Spiess, 1999), an "anxiolytic" effect was defined as a reduction in behaviors evoked by a stressor, whereas an "anxiogenic" effect was defined as an augmentation of such behaviors.

Tail Pinch. An increase in the following behaviors was interpreted as evidence of anxiogenesis in the tail pinch condition: eating, gnawing, oral behavior directed at food, oral behavior not directed at food, grooming, vocalizations, and defecation.

Open Field. In the open field, an increase in freezing behavior was regarded as evidence of anxiogenesis. Increases in line crosses and rearing were considered anxiolytic effects. The occurrence of head and body shakes was regarded as a nonspecific effect of 5-HT ligands unrelated to the stress response.

Rotarod. Rotarod performance was used to assess general motor function and was considered to be unrelated to the stress response.

Recording Data. Any behavior involving a timed measure was recorded to the nearest hundredth of a second, with the exception of the rotarod, which was measured to the tenth of a second. Any variable measured according to weight, such as eating and gnawing, was measured in grams and recorded to the nearest hundredth of a gram.

Statistical Analyses

Data collected in the tail pinch and the open field were analyzed using Multivariate Analysis of Variance (MANOVA). Data collected on the rotarod were analyzed separately by univariate analysis (ANOVA). Rotarod data were not included in the MANOVA because some animals were unable to meet the training criterion, resulting in a lower number of data points relative to other behaviors recorded in the tail pinch and open field.

Results

An analysis of tail pinch data revealed that oral behavior directed at food (OB w/ food) was the predominant behavioral response to tail pinch stress. On average, animals spent approximately 80 of a possible 240 seconds engaged in oral behavior directed at food. However, 36% of the animals did not exhibit any OB w/ food under saline conditions. Inclusion of these zeroes in our initial analysis resulted in an inflated error term, and a concomitant reduction of statistical significance in our analyses. In experiments 4 and 5, this inflation led to a diminution of statistical significance with regard to DOI relative to saline. Thus, a post-hoc exclusionary criterion of 2 seconds was set for OB w/ food in the saline condition. To be included in the secondary (post-culling) analyses, an animal must have exhibited more than 2 seconds of OB w/ food in the saline condition. Animals not meeting this criterion were culled from further analyses. Results of both analyses are included in the following sections. For the sake of simplicity, excluded animals will be referred to as *non-responders* in the subsequent discussion.

Statistical Analysis

Statistical analyses were conducted using SPSS (Statistical Package for the Social Sciences, V. 10.0 for Windows). Tail pinch and open field behaviors were analyzed by MANOVA. Rotarod data were analyzed separately by ANOVA.

Primary Analysis. Prior to exclusion of non-responders, data were analyzed to determine if drug condition significantly affected behavioral responses to stress. The results of each MANOVA are summarized in the following sections. In each experiment, drug condition was the independent variable. Dependent variables were outlined in previous sections and are listed individually in the following tables. Prior to application

of the exclusionary criterion, *n* for each experiment was as follows: 15 (Exp. 1), 16 (Exp 2), 16 (Exp. 3), 15 (Exp. 4), and 16 (Exp. 5).

Secondary Analysis. After implementing an exclusionary criterion, *n* for each experiment was reduced to the following: 12 (Exp. 1), 10 (Exp. 2), 10 (Exp. 3), 8 (Exp. 4), and 10 (Exp. 5). In an effort to achieve roughly equivalent *n* in each experiment, animals were added to supplement experiments 4 and 5, bringing the final *n* in these experiments to 11 and 13, respectively. These animals were subject to identical methodological considerations given prior groups and were assigned to first-day drug conditions in a manner designed to compensate for excluded data.

Experiment 1

Primary Analysis. Experiment 1 established a dose-response curve for DOI. The overall MANOVA was significant [$F(39, 107)=2.84$, $p=.000$], using Wilk's criterion. The resulting univariate ANOVAs for each dependent variable are included in Table 3. Variables that differed significantly across at least one experimental condition are denoted with an asterisk.

Secondary Analysis. As was the case in the primary analysis, the overall MANOVA for the four experimental conditions was found to be significant [$F(39,96)=2.38$, $p=.000$]. The secondary univariate analysis is summarized in Table 3.

Table 3: Experiment 1 Univariate ANOVAs by Behavior

		Primary		Secondary	
Behavior	df	F	**p**	F	**p**
Eating	3	1.77	0.16	1.95	0.14
Gnawing	3	0.60	0.62	0.65	0.59
Oral Behavior Directed at Food*	3	5.01	0.004	7.12	0.001

Table cont.

Oral Behavior Not Directed at Food	3	0.23	0.88	1.64	0.19
Grooming	3	1.35	0.27	2.52	0.07
Vocalizations	3	1.63	0.19	1.00	0.40
Boli (Tail Pinch)	3	0.67	0.57	0.73	0.54
Line Crosses	3	2.67	0.06	2.70	0.057
Rearing*	3	12.05	0.000	10.60	0.000
Head Shakes*	3	6.65	0.001	5.21	0.002
Body Shakes	3	2.15	0.10	2.20	0.10
Boli (Open Field)	3	0.30	0.82	0.00	1.00
Freezing	3	2.25	0.09	2.31	0.09

Experiment 1 post hoc analysis. Primary post hoc analyses revealed that DOI altered responding in the following three behavioral variables: oral behavior directed at food, rearing, and head shakes. Secondary analysis revealed significant findings for the same three variables: oral behavior directed at food, rearing, and head shakes.

Oral behavior directed at food (Figures 5 and 6). Primary and secondary analysis generated statistically similar findings with respect to oral behavior directed at food. OB w/ food was reduced by the middle ($p_{primary}$=.010; $p_{secondary}$=.003) and high ($p_{primary}$=.002;

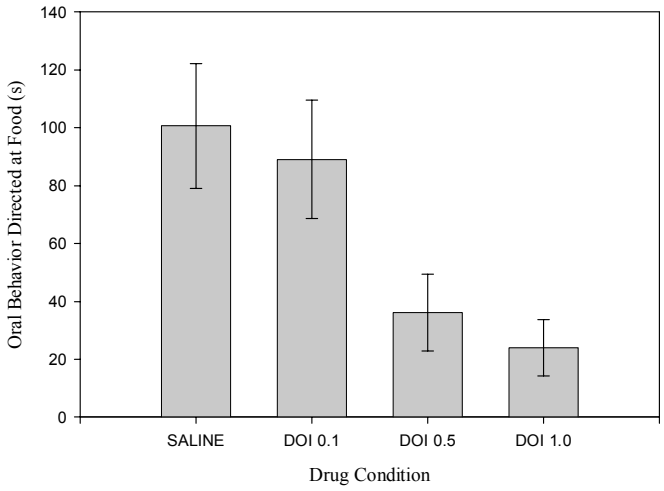

Figure 5: OB w/ Food (Exp. 1 Primary Analysis)

$p_{secondary}$=.000) doses of DOI, relative to saline. OB w/ food was also reduced, relative to low-dose DOI, by middle ($p_{primary}$=.032; $p_{secondary}$=.012) and high ($p_{primary}$=.009; $p_{secondary}$=.002) doses of DOI.

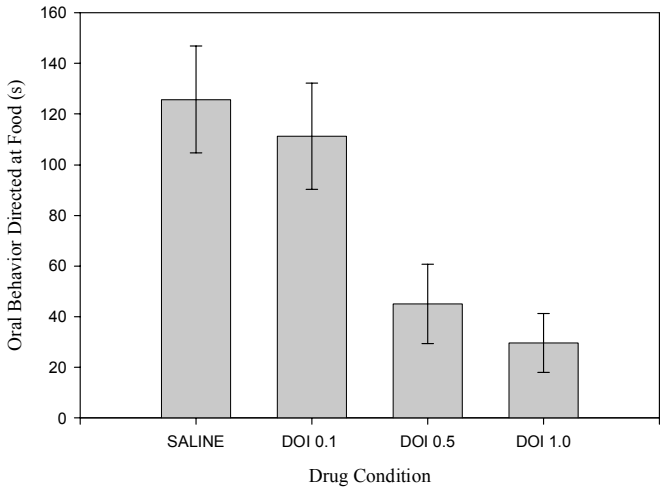

Figure 6: OB w/ Food (Exp. 1 Secondary Analysis)

Rearing (Figures 7 and 8). As was the case with OB w/ food, primary and secondary analyses of rearing yielded similar results. Rearing was reduced by middle ($p_{primary}$=.000; $p_{secondary}$=.001) and high ($p_{primary}$=.001;$p_{secondary}$=.001*)* doses of DOI, relative to saline.

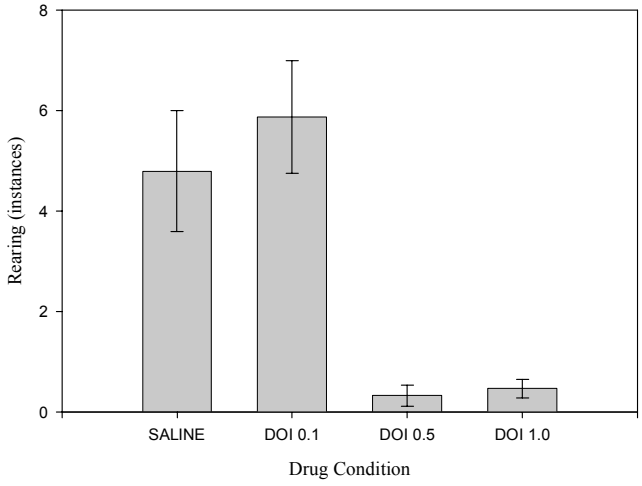

Figure 7: Rearing (Exp. 1 Primary Analysis)

Rearing was also reduced by middle ($p_{primary}$=.000; $p_{secondary}$=.000) and high ($p_{primary}$=.000; $p_{secondary}$=.000) doses of DOI, relative to low-dose DOI.

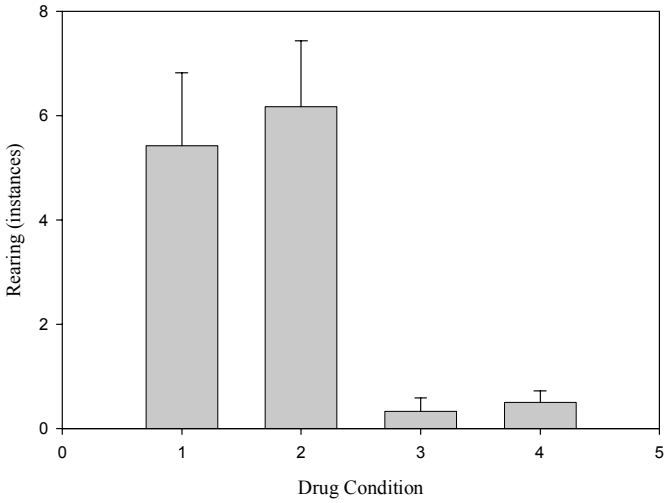

Figure 8: Rearing (Exp. 1 Secondary Analysis)

Head Shakes (Figures 9 and 10). Head shaking was increased by the middle dose of DOI relative to saline ($p_{primary}$=.000; $p_{secondary}$=.000), low-dose DOI ($p_{primary}$=.001; $p_{secondary}$=.003), and high-dose DOI ($p_{primary}$=.003; $p_{secondary}$=.007). Frequency of head shakes

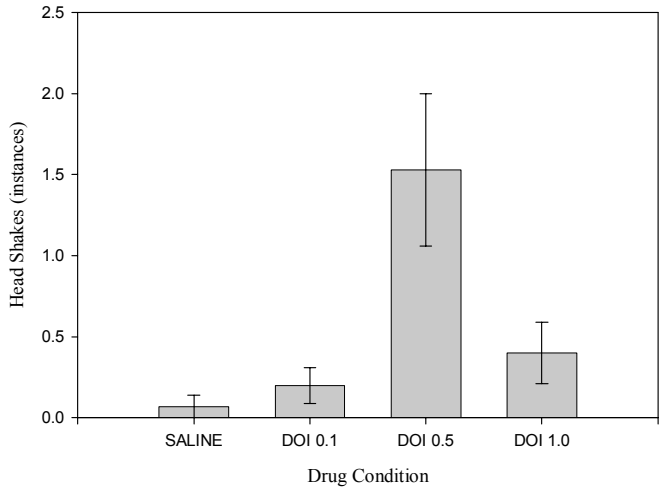

Figure 9: Head Shakes (Exp. 1 Primary Analysis)

was not affected by the low or high doses of DOI, relative to controls, in either the primary or secondary analyses.

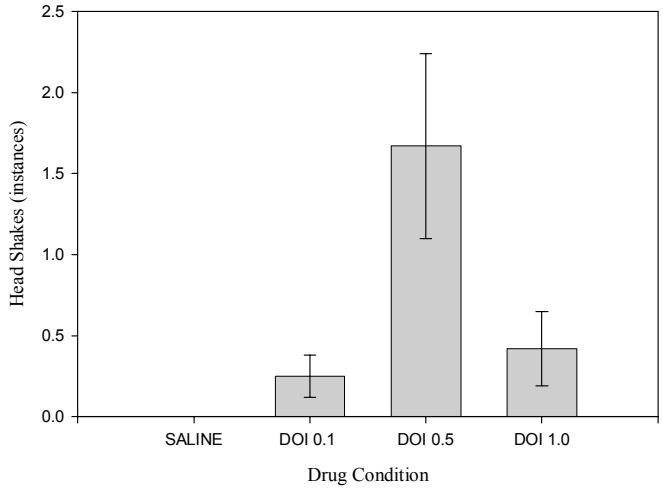

Figure 10: Head Shakes (Exp.1 Secondary Analysis)

Experiment 2

Primary/secondary Analyses. Experiment 2 examined the dose-response profile of the 5-HT_{2A} antagonist, spiperone. Primary analysis by MANOVA revealed that spiperone did not significantly alter behavioral responding when administered alone [F(36,145)=1.35; p=.11]. This was also the case for the secondary MANOVA, [F(33,77)=1.17, *p*=.284.

Experiment 3

Primary/secondary Analyses. Experiment 3 evaluated the possible behavioral effects of the 5-HT_{2C} antagonist, SDZ SER 082. As with spiperone, 5-HT_{2C} antagonism alone did not affect behavioral responses to stress based on the primary analysis by MANOVA [F(33,148)=1.02, p =.45]. Similarly, the secondary MANOVA was not statistically significant, [F(33,77)=1.01, p=.466.

Experiment 4

Dose Selection. Experiment 4 examined the possibility that the effects of DOI on stress-induced behavior might be reduced using a single selective antagonist. Doses for DOI, spiperone ($_{2A}$ antagonist), and SDZ SER 082 ($_{2C}$ antagonist) were chosen based on findings from Experiments 1, 2, and 3. In the case of DOI, the middle dose (0.5 mg/kg) was selected based on the fact that this dose of DOI produced behavioral effects that were statistically indistinguishable from high-dose DOI. Therefore, it was reasonable to conclude that behaviors affected by the middle dose of DOI had a greater likelihood of being antagonized in Experiment 4.

Appropriate doses of spiperone and SDZ SER 082 were determined based on findings from Experiments 2 and 3. Because neither drug was shown to alter behavioral responding when administered alone, the highest dose (1.0 mg/kg) for each drug was selected.

Analyses. The primary MANOVA for the four experimental conditions was significant, [F(39,131)=3.24, p=.000], as was the secondary MANOVA [F(36,86)=1.82, *p*=.012]. The resulting univariate ANOVAs are included in Table 4 below. Behaviors that differed significantly across at least one experimental condition are denoted with an asterisk.

Table 4: Experiment 4 Univariate ANOVAs by Behavior

		Primary		Secondary	
Behavior	df	F	**p**	F	**p**
Eating	3	0.25	0.86	0.60	0.62
Gnawing	3	0.73	0.54	0.05	0.99
Oral Behavior Directed at Food*	3	2.24	0.09	4.00	0.01

Table cont.

Oral Behavior Not Directed at Food	3	1.73	0.17	0.80	0.50
Grooming	3	2.18	0.10	0.37	0.77
Vocalizations	3	1.85	0.15	2.03	0.13
Boli (Tail Pinch)	3	0.71	0.55	0.88	0.46
Line Crosses*	3	7.52	0.00	3.26	0.03
Rearing*	3	5.65	0.00	2.66	0.06
Head Shakes*	3	7.38	0.00	4.44	0.00
Body Shakes	3	1.00	0.40	-	-
Boli (Open Field)	3	18.83	0.59	0.50	0.69
Freezing*	3	0.64	0.00	5.07	0.00

Experiment 4 Post Hoc Analyses. Primary and secondary post hoc analyses generated different results for Experiment 4. Primary post hoc analyses revealed that drug conditions altered four behavioral variables: line crosses, rearing, head shakes, and freezing. Secondary post hoc analyses also indicated that drug conditions affected responding across four behavioral variables. However, these four variables differed slightly from the primary post hoc analyses: oral behavior directed at food, line crosses, head shakes, and freezing.

Oral Behavior Directed at Food (Figure 11). Secondary analysis revealed the OB w/food was reduced, relative to saline, by DOI (p=.007), DOI + spiperone (p=.008), and

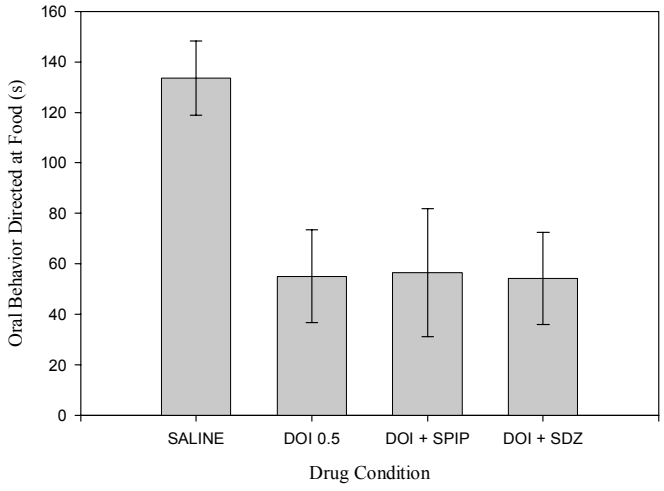

Figure 11: OB w/ Food (Exp. 4 Secondary Analysis)

DOI + SDZ SER 082 (p=.007). That is, neither antagonist reversed the DOI-induced reductions in oral behavior.

Line crosses (Figures 12 and 13). In both the primary and secondary analyses, injections of DOI + SDZ SER 082 resulted in decreased line crossing relative to saline ($p_{primary}$=.000; $p_{secondary}$=.012). In the primary analysis, injections of DOI + SDZ SER 082 were also shown to decrease line crossing relative to DOI alone (p=.026). Primary analysis also showed that DOI + spiperone increased line crossing relative to DOI (p=.044).

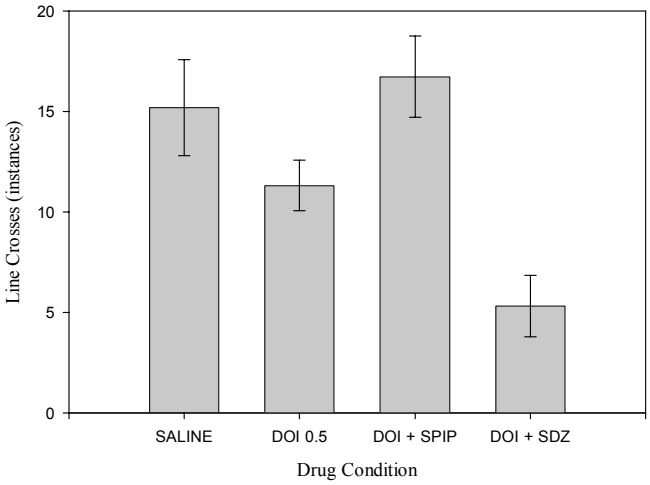

Figure 12: Line Crosses (Exp. 4 Primary Analysis)

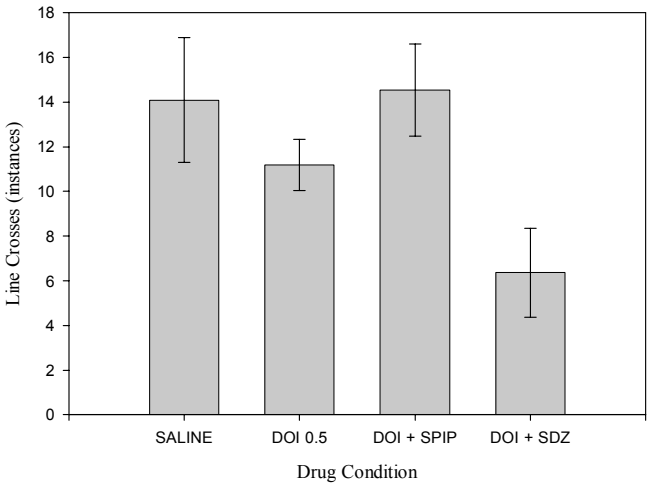

Figure 13: Line Crosses (Exp. 4 Secondary Analysis)

Rearing (Figure 14). Primary analysis indicated that injections of DOI reduced rearing, relative to saline (p=.006), as did DOI + spiperone (p=.008), and DOI + SDZ SER 082 (p=.000). Neither antagonist was able to reverse the DOI-induced reduction of rearing.

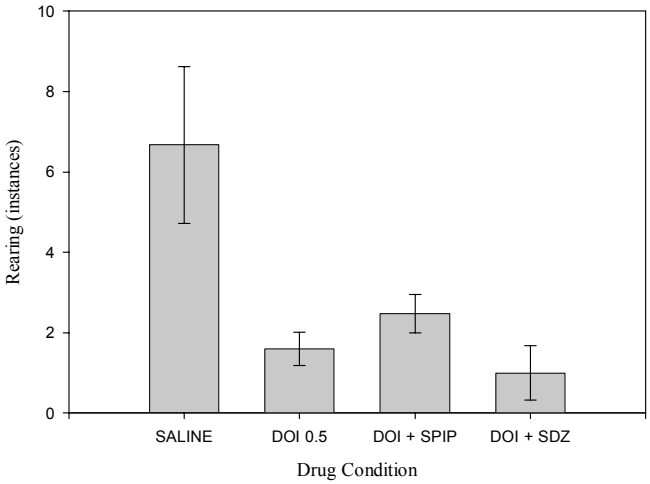

Figure 14: Rearing (Exp. 4 Primary Analysis)

Head shakes (Figures 15 and 16). In the primary and secondary analyses, DOI increased head shakes relative to both saline ($p_{primary}$=.006; $p_{secondary}$=.004) and DOI + SDZ SER 082 ($p_{primary}$=.006; $p_{secondary}$=.004), as did DOI + spiperone (p=.000), which suggests that the 5-HT_{2C} antagonist was able to reverse DOI-induced head shaking. Both

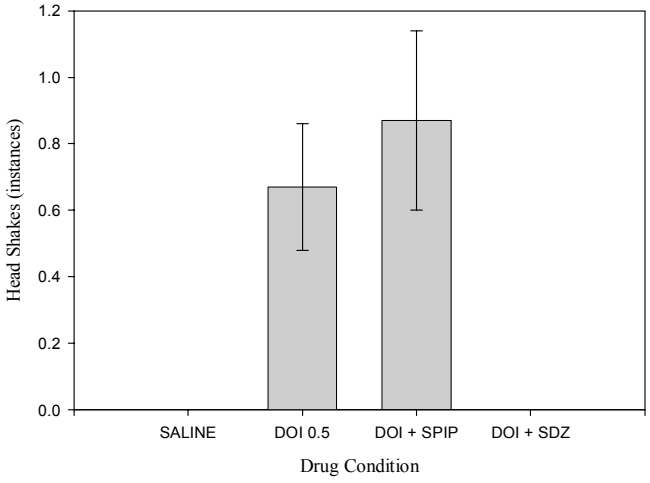

Figure 15: Head Shakes (Exp. 4 Primary Analysis)

primary and secondary analyses indicated spiperone was ineffective in blocking DOI-induced head shaking.

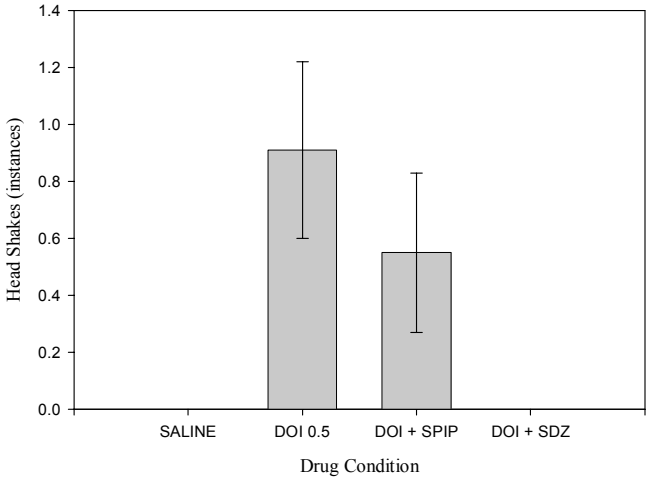

Figure 16: Head Shakes (Exp. 4 Secondary Analysis)

Freezing (Figures 17 and 18). Primary and secondary analyses generated the same findings with respect to freezing. Injections of DOI + SDZ SER 082 resulted in increased freezing relative to saline ($p_{primary}$=.000; $p_{secondary}$=.006), DOI ($p_{primary}$=.000; $p_{secondary}$=.001), and DOI + spiperone ($p_{primary}$=.000; $p_{secondary}$=.004).

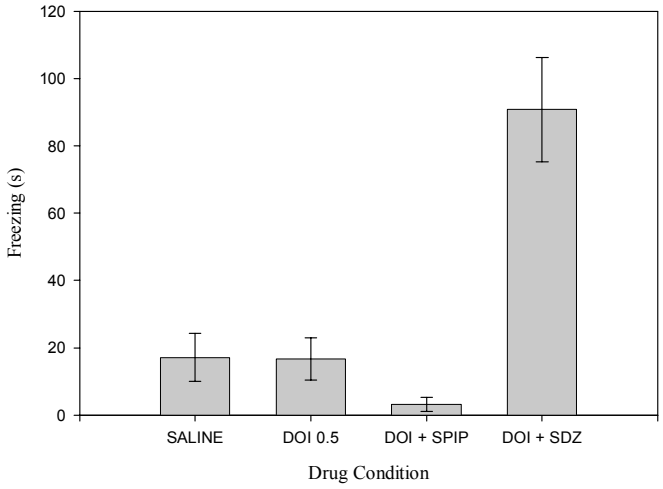

Figure 17: Freezing (Exp. 4 Primary Analysis)

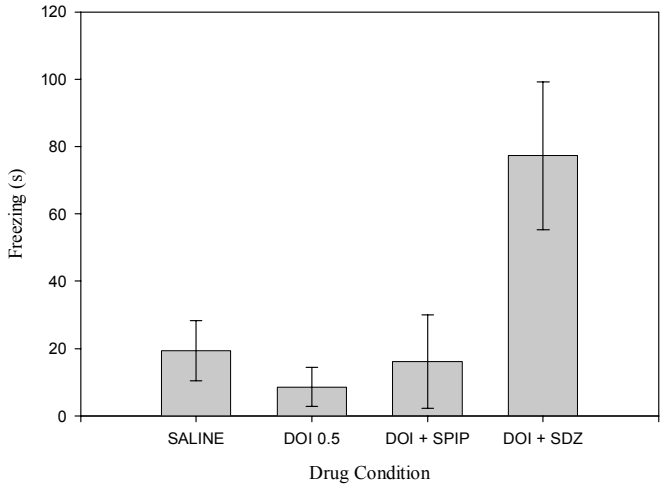

Figure 18: Freezing (Exp. 4 Secondary Analysis)

Experiment 5

Experiment 5 examined the effects of DOI on stress-induced behavior when co-administered with a combination of 5-HT_{2A} and 5-HT_{2C} antagonists. The overall primary MANOVA was significant F(39,142)=7.48, p=.000], as was the secondary MANOVA [F(39,107)=3.735, *p*=.000]. As before, the resulting univariate ANOVAs from each analysis are included in Table 5 below. Significant variables are denoted with an asterisk.

Table 5: Experiment 5 Univariate ANOVAs by Behavior

		Primary		Secondary	
Behavior	df	F	**p**	F	p
Eating	3	0.57	0.63	0.85	0.48
Gnawing*	3	0.94	0.43	2.73	0.05
Oral Behavior Directed at Food*	3	2.22	0.10	6.24	0.00
Oral Behavior Not Directed at Food*	3	11.44	0.00	5.15	0.00
Grooming*	3	3.74	0.02	1.07	0.37
Vocalizations	3	2.01	0.12	0.11	0.95
Boli (Tail Pinch)	3	0.84	0.48	0.43	0.73

Table cont.

Line Crosses*	3	24.47	0.00	17.73	0.00
Rearing*	3	18.87	0.00	15.60	0.00
Head Shakes*	3	17.55	0.00	7.67	0.00
Body Shakes*	3	9.92	0.00	3.49	0.02
Boli (Open Field)	3	52.64	0.09	1.64	0.19
Freezing*	3	2.24	0.00	32.36	0.00

Experiment 5 Post Hoc Analysis. Primary post hoc analysis revealed that drug conditions altered responding across seven behavioral variables: oral behavior without food, grooming, line crosses, rearing, head shakes, body shakes, and freezing. Secondary post hoc analysis revealed that drug conditions significantly altered responding in eight behavioral variables: gnawing, oral behvaior directed at food, oral behavior not directed at food, line crosses, rearing, head shakes, body shakes, and freezing.

Gnawing (Figure 19). Secondary analysis showed that gnawing was reduced, relative to saline, by spiperone + SDZ SER 082 (p=.038) and DOI + spiperone + SDZ SER 082 (p=.047).

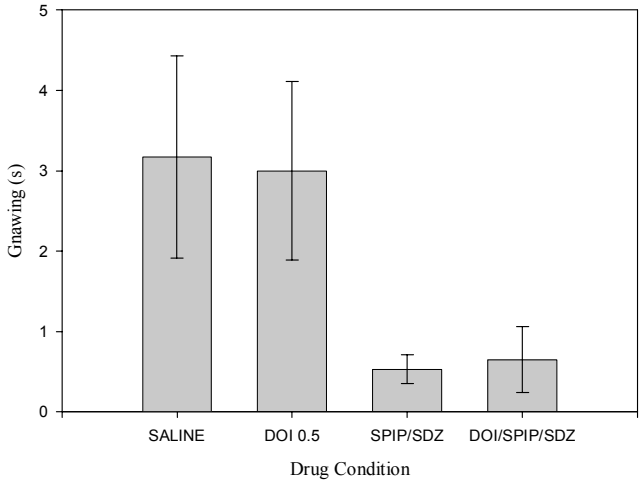

Figure 19: Gnawing (Exp. 5 Secondary Analysis)

Oral Behavior Directed at Food (Figure 20). Based on secondary analysis, oral behavior directed at food was reduced, relative to controls, by DOI alone (p=.020), DOI + spiperone (p=.002), and DOI + spiperone + SDZ SER 082 (p=.004).

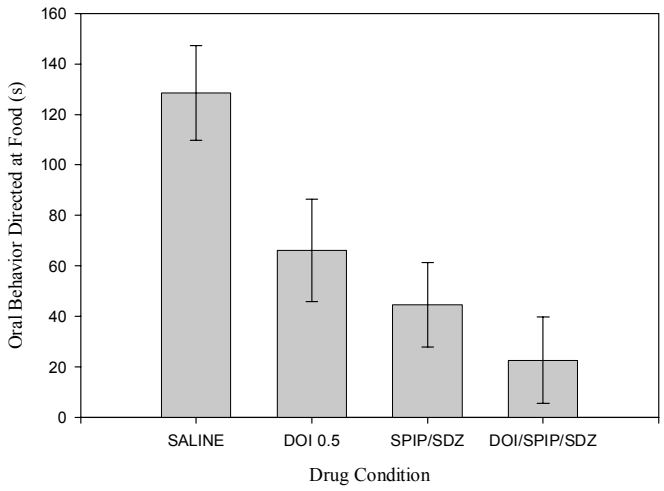

Figure 20: OB w/ Food (Exp. 5 Secondary Analysis)

Oral Behavior Not Directed at Food (Figures 21 and 22). Both analyses generated similar findings with respect to oral behavior not directed at food. OB w/o food was reduced, relative to saline, by spiperone + SDZ SER 082 ($p_{primary}$=.000; $p_{secondary}$=.016) and DOI + spiperone + SDZ SER 082 ($p_{primary}$=.000; $p_{secondary}$=.009). This behavior was

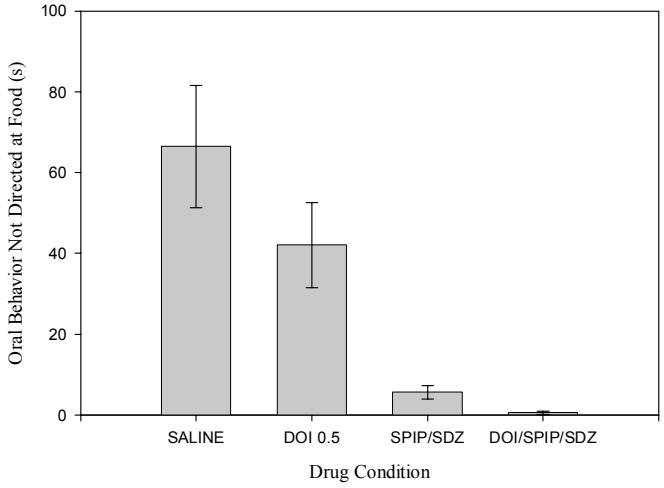

Figure 21: OB w/o Food (Exp. 5 Primary Analysis)

also significantly reduced, relative to DOI alone, by spiperone + SDZ SER 082 ($p_{primary}$=.007; $p_{secondary}$=.007), and DOI + spiperone + SDZ SER 082 ($p_{primary}$=.002; $p_{secondary}$=.004).

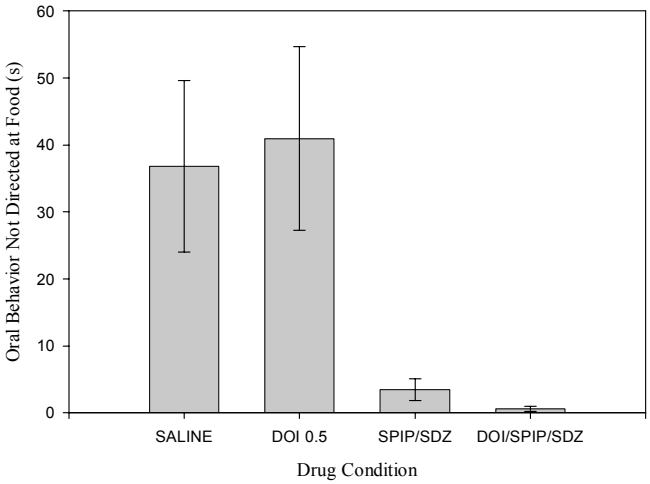

Figure 22: OB w/o Food (Exp. 5 Secondary Analysis)

Grooming (Figure 23). Primary analysis revealed that spiperone + SDZ SER 082 decreased grooming, relative to saline (p=.004), as did DOI + spiperone + SDZ SER 082 (p=.007).

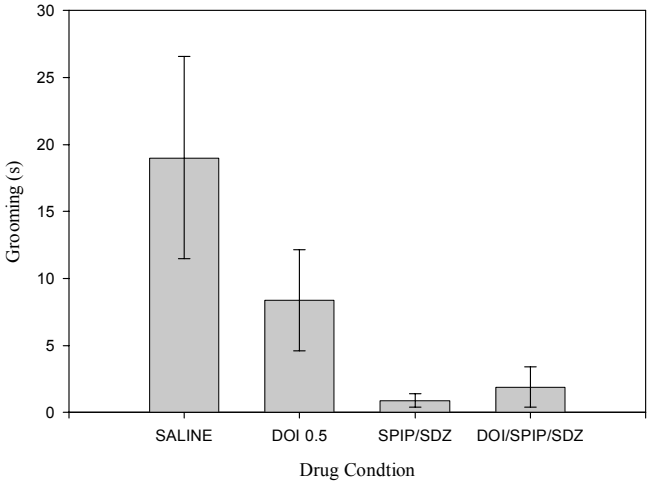

Figure 23: Grooming (Exp.5 Primary Analysis)

Line Crossing (Figures 24 and 25). Each analysis generated identical findings with respect to line crossing. Line crossing was significantly reduced, relative to controls, by

spiperone + SDZ SER 082 ($p_{primary}$=.000; $p_{secondary}$=.000) and DOI + spiperone + SDZ SER 082 ($p_{primary}$=.000; $p_{secondary}$=.000). Line crossing was also significantly reduced, relative to DOI alone, by spiperone + SDZ SER 082 ($p_{primary}$=.000; $p_{secondary}$=.000) and DOI + spiperone + SDZ SER 082 ($p_{primary}$=.000; $p_{secondary}$=.000).

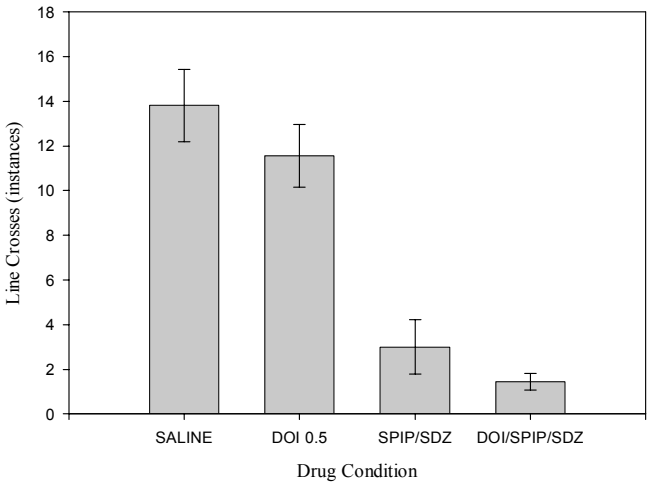

Figure 24: Line Crosses (Exp. 5 Primary Analysis)

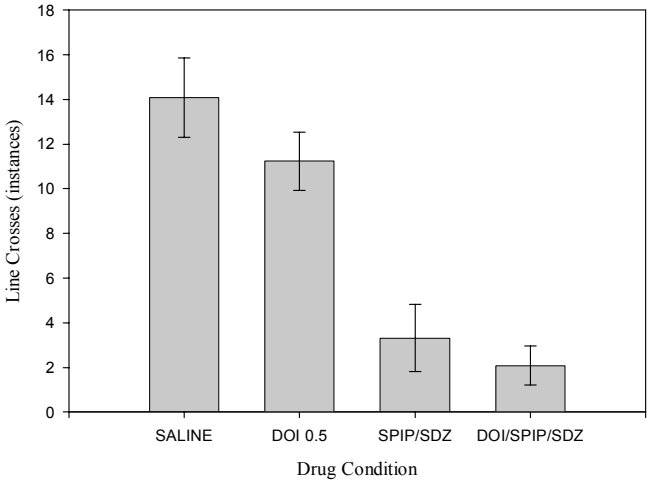

Figure 25: Line Crosses (Exp. 5 Secondary Analysis)

Rearing (Figures 26 and 27). Both analyses indicated that rearing was reduced, relative to saline, by DOI ($p_{primary}$=.000; $p_{secondary}$=.000), spiperone + SDZ SER 082

($p_{primary}$=.000; $p_{secondary}$=.000), and DOI + spiperone + SDZ SER 082 ($p_{primary}$=.000; $p_{secondary}$=.000).

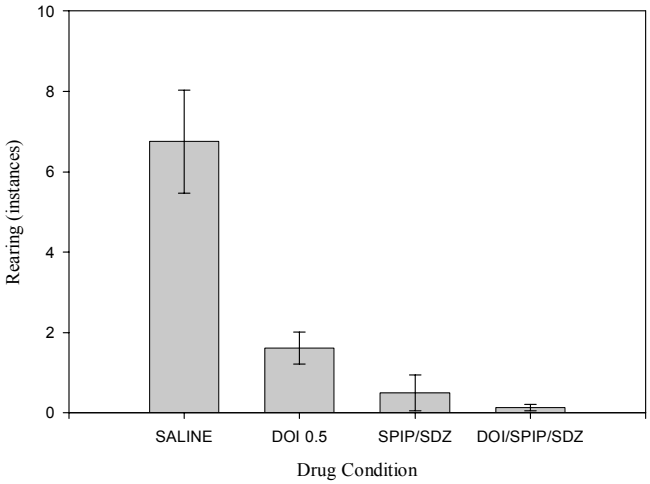

Figure 26: Rearing (Exp. 5 Primary Analysis)

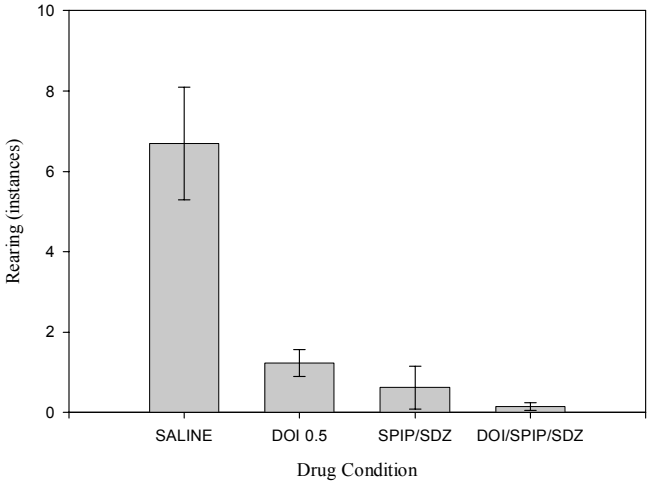

Figure 27: Rearing (Exp. 5 Secondary Analysis)

Head Shakes (Figures 28 and 29). Primary and secondary analyses revealed similar results with respect to head shakes. Injections of DOI increased head shakes relative to saline ($p_{primary/secondary}$=.000), spiperone + SDZ SER 082 ($p_{primary/secondary}$=.000), and DOI + spiperone + SDZ SER 082 ($p_{primary/secondary}$=.000).

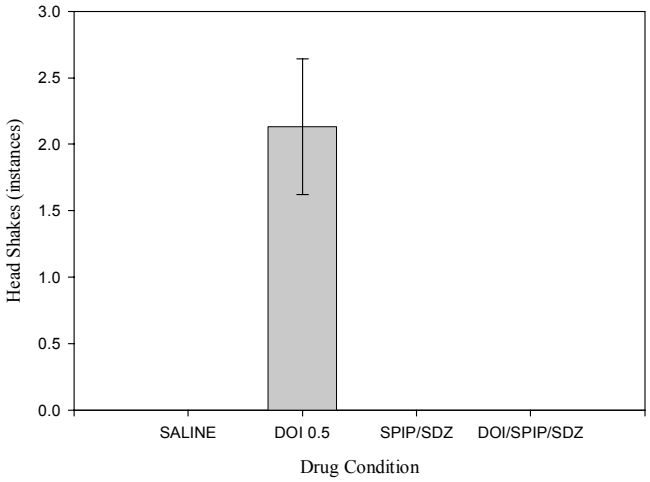

Figure 28: Head Shakes (Exp. 5 Primary Analysis)

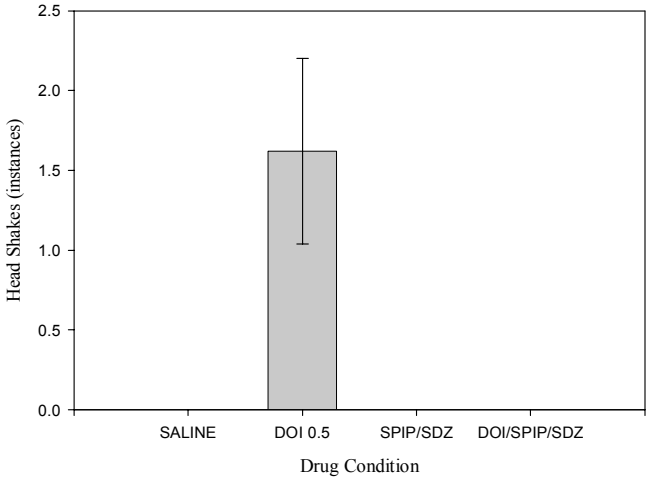

Figure 29: Head Shakes (Exp. 5 Secondary Analysis)

Body Shakes (Figures 30 and 31). Once again, the primary and secondary analyses yielded similar findings with respect to open field behavior. DOI increased body shakes relative to saline ($p_{primary}$=.000; $p_{secondary}$=.008), spiperone + SDZ SER 082 ($p_{primary}$=.000; $p_{secondary}$=.031), and DOI + spiperone + SDZ SER 082 ($p_{primary}$=.000; $p_{secondary}$=.008). These findings suggest that DOI-induced head and body shakes can be attenuated by a combination of 5-$HT_{2A/2C}$ antagonists. However, based on data from the rotarod, tail pinch

and other open field behaviors, it may simply be the case that the administration of multiple antagonists resulted in sedation. This issue will be discussed in later sections.

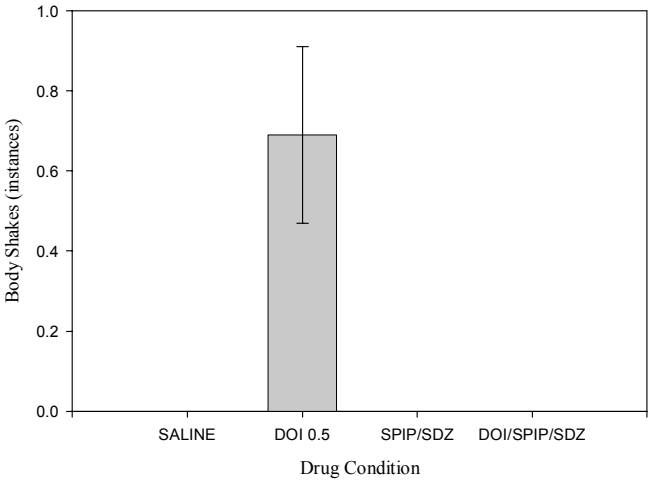

Figure 30: Body Shakes (Exp. 5 Primary Analysis)

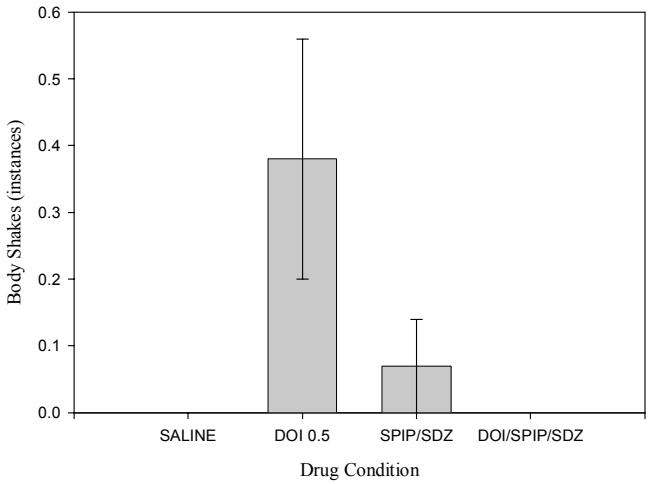

Figure 32: Body Shakes (Exp. 5 Secondary Analysis)

Freezing (Figures 33 and 34). Primary and secondary analyses produced similar findings in the case of freezing behavior. Spiperone + SDZ SER 082 increased freezing relative to saline ($p_{primary/secondary}$=.000) and DOI ($p_{primary/secondary}$=.000). DOI + spiperone + SDZ SER 082 also increased freezing relative to saline ($p_{primary/secondary}$=.000) and DOI ($p_{primary/secondary}$=.000).

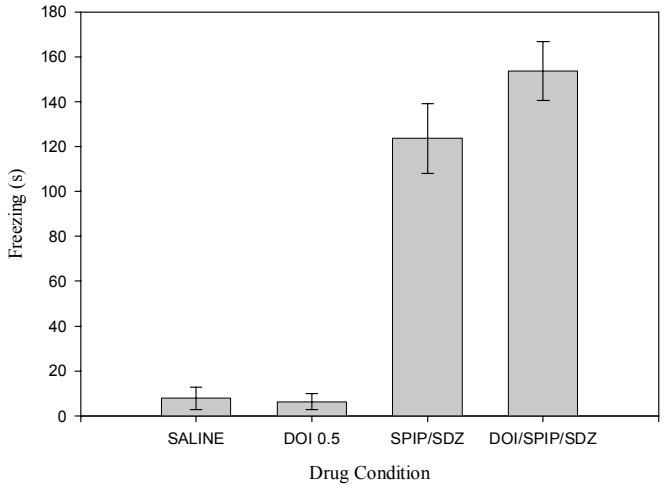

Figure 33: Freezing (Exp. 5 Primary Analysis)

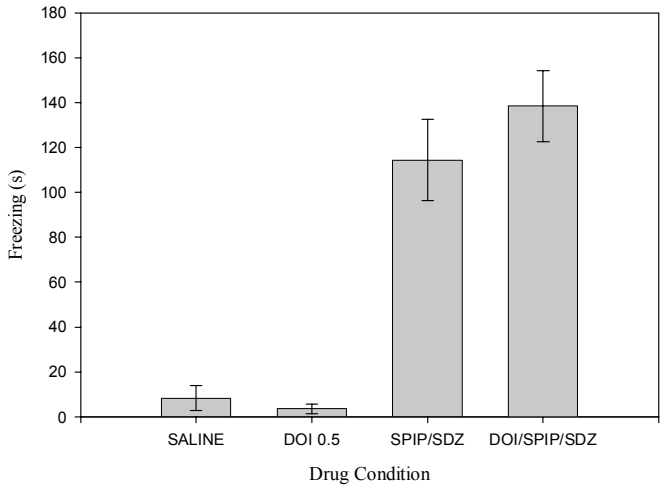

Figure 34: Freezing (Exp. 5 Secondary Analysis)

Rotarod Data. In all five experiments, ANOVAs for rotarod data failed to achieve statistical significance. However, in Experiment 5, aberrations were seen in animals injected with spiperone + SDZ SER 082, as well as DOI + spiperone + SDZ SER 082. In both instances, animals' time spent on the rotarod was decreased (nonsignificantly) relative to DOI alone and controls. It is worthy of note that *any* deviation from normal rotarod performance was exceedingly rare in this experiment. Additionally, animals in

aforementioned groups exhibited behavior suggestive of sedation in both the tail pinch and open field. These issues will be addressed in the following discussion.

Discussion

The current study was conducted in an effort to further examine the influence of serotonergic ligands on stress responding in rats. Eight hypotheses were tested over the course of five experiments. The following discussion will examine each of the hypotheses based on findings from the secondary analyses presented in the previous section.

Hypothesis 1: The Non-selective 5-HT_2 Agonist, Injected Subcutaneously, Will Decrease Tail Pinch-induced Stress Responding in Rats

Both the middle and high doses of DOI were shown to reduce tail pinch-induced oral behavior directed at food (Exp. 1,4, and 5). This replicates the previously published finding from our laboratory that DOI decreases stress-evoked oral behavior (Hawkins, et al., 2002). The effects of DOI on oral behavior can be interpreted in various ways. The current paper will examine the findings in the context of published work regarding the effects of DOI in various models of stress and anxiety. The possibility that the behavioral effects of DOI are the result of increased satiety, or of a nonspecific malaise, will also be examined.

DOI's Effect on Stress Responding. Results of Experiment 1, 4, and 5 indicate that DOI dose-dependently reduces stress-evoked oral behavior directed at food. This finding is consistent with much of the existing literature regarding 5-$HT_{2A/2C}$ agonists and their effects on stress and anxiety. Although these studies employ different models of stress, it is possible to generalize the findings in terms of the general effects of 5-$HT_{2A/2C}$ agonists on paradigm-specific behavior.

Peripheral injections of DOI have been shown to increase punished responding in the four-plate test, a finding indicative of decreased sensitivity to physical stress (Dhonnchadha, Hascoet, Jolliet, and Bourin, 2003). Central and peripheral administration of 5-HT_2

agonists have also been shown to decrease escape behavior in models involving aversive stimuli, such as direct stimulation of the dorsal periaqueductal gray and exposure to the elevated T-plus maze (de Paula Soares, 2004; Mora, Netto, and Graeff, 1997; Zangrossi, et al., 2001; Graeff, et al., 1993). These findings, coupled with a replicated demonstration that DOI reduces stress-evoked oral behavior in our laboratory, suggest that 5-$HT_{2A/2C}$ agonists decrease behavioral responding to physical stress.

DOI and Satiety. It is possible that the effects of DOI are the result of an alteration in behavioral satiety sequences in rodents. Although the present study did not compare eating in tail pinch animals versus unstressed controls, other studies have demonstrated that tail pinch stress increases ingestive behavior in rodents (Levine and Morley, 1981; Koch and Bodnar, 1993; Szechtman and Hall, 1980; Heinrichs, et al., 1992). Stress-induced increases in food intake are blockable with central and peripheral administration of DOI and other 5-$HT_{2A/2C}$ agonists (Currie, Coiro, Niyomchai, Lira, and Farahmand, 2002; Currie, Saxena, and Tu, 1999; Currie and Coscina, 1997). These behavioral effects have been attributed to the ability of 5-HT_2 agonists to activate the HPA axis, thereby slowing gastric emptying and inducing satiety (Raghavendra and Kulkarni, 2000; Ferrari, Pelloni, and Guiliani, 1992; Baker, Duggan, Barber, and Booth, 1998; Francis, Critchley, Dourish, and Cooper, 1995).

Although the current study consistently demonstrated that DOI reduces oral behavior directed at food, there was no statistically significant DOI-induced reduction in eating or gnawing. Thus, the suggestion that DOI induced satiety in the current study must be made cautiously. We did observe a dose-dependent trend toward reduced eating following DOI injections in Experiment 1. Low, medium, and high doses of DOI reduced eating, relative to saline, by 10, 70, and 71%, respectively. Additionally, previous work in our lab has

demonstrated that DOI dose-dependently reduces eating and gnawing (Hawkins, et al., 2002).

Drug-induced Malaise. 5-HT and related compounds are known to induce malaise, a behavioral profile typified by nausea, tremor, and fatigue (DeVry, Eckel, Kuhl, and Schreiber, 2000). This is a potential confound when interpreting the effects of DOI on ingestion and oral behavior, as the anorectic behavioral profile seen in these studies may indicate nausea, rather than a decrease in anxiety (DeVry, et al., 2000). A study involving the co-administration of DOI and an anti-emetic compound might be of utility in determining if nausea is responsible for the behavioral effects produced by 5-HT_2 agonists.

Hypothesis 2: The Effect of DOI On Tail Pinch-induced Stress Responding Will be Reduced by Peripheral Co-administration of the Selective 5-HT_{2A} Antagonist, Spiperone

Experiment 4 examined the effects of co-administration of a single antagonist with DOI. Spiperone did not alter the behavioral effects of DOI in the tail pinch condition. This finding is inconsistent with previous reports that spiperone blocks DOI-induced hypophagia in tail-pinched rats (Samarghandian, Ohata, Yamauchi, and Shibasaki, 2003). It should be noted that the Samarghandian, et al. study employed intracerebroventricular injections of spiperone. As such, it is possible that the permeability of the blood-brain to spiperone may be a factor moderating the drug's effects. Although spiperone is known to cross the BBB, definitive data regarding the degree to which spiperone crosses this barrier are not currently available (Herberg, and Wishart, 1980).

Hypothesis 3: The Effect of DOI on Tail Pinch-induced Stress Responding Will be Reduced by Peripheral Co-administration of the Selective 5-HT_{2C} Antagonist, SDZ SER 082

Experiment 4 failed to produce any evidence that SDZ SER 082 altered behavioral responding to tail pinch and DOI injection. Although relatively few studies employing SDZ SER 082 have been published, this finding is inconsistent with reports that SDZ SER 082 blocks 5-HT-induced behavior in certain models of stress (de Paula Soares and Zangrossi, 2004). Further evidence of SDZ SER 082's ability to alter oral and ingestive behavior has been demonstrated in studies involving 5-HT_2-selective compounds (Castro, et al., 2003, Castro, et al., 2002, Currie, Coiro, Nyomchai, Lira, and Farahmand, 2002). However, none of these studies examined *stress-evoked* oral behavior, as was the case in the current study. Additionally, these studies involved central, rather than peripheral, injections of 5-HT_2 ligands. Thus, the possibility exists that the effects of SDZ SER 082 are limited by the compound's ability to cross the BBB. Still, SDZ SER 082 has been shown to alter behavioral responding when administered peripherally (Goudie, Smith, Taylor, Taylor, and Tricklebank, 1998; Mora, Netto, and Graeff, 1997). While 5-HT_{2C} receptors are known to exist in the periphery, it is more plausible that the effects of SDZ SER 082 on anxiety and stress are centrally-mediated, suggesting permeability of the BBB to spiperone (Mora, Netto, and Graeff, 1997). Conclusive data regarding the permeability of the BBB to SDZ SER 082 are not currently available, however.

Hypothesis 4: Spiperone and SDZ Will Have No Effect on Stress Responding When Administered Alone

Experiments 2 and 3 examined the effects of spiperone and SDZ SER 082, injected individually. Both primary and secondary MANOVAs were not significant, indicating that

neither compound altered behavioral responding when injected alone. This is compatible with previous findings that 5-HT_{2A} antagonists are ineffective in altering anxious behavior evoked by stressors (Gleeson, Ahlers, Mansbach, Foust, and Barrett, 1989; Griebel, Perrault, and Sanger, 1997; Setem, Pinheiro, Motta, Morato, and Cruz, 1999, Bourin and Hascoet, 2003). Conversely, various studies have demonstrated that 5-HT_{2A} and 5-HT_{2C} antagonists, injected alone, decrease stress responding in various rodent models (Stutzmann, et al., 1991, Motta, Maisonnette, Morato, Castrechini, and Brandao, 1992, Costall and Naylor, 1995; Mora, et al., 1997; Graeff, Netto, and Zangrossi, 1998; Gacsalyi, et al., 1997, Griebel, et al., 1997; Dekeyne, Denorme, Monneyron, and Millan, 2000). However, findings that these antagonists increase stress responding have been reported also (Olivier, et al., 1998, Setem, et al., 1999, Maisonette, et al., 2000). The discrepancy in these findings may be partially attributable to the nature of the stressor employed in these studies, an issue that has been addressed in the Deakin and Graeff theory of 5-HT and anxiety (discussed below).

Hypothesis 5: Simultaneous Administration of Spiperone and SDZ SER 082 Will Result in Greater Antagonism of DOI's Effects Than That of Either Antagonist Administered Alone

Experiment 5 examined the possibility that co-administration of spiperone and SDZ SER 082 would alter DOI-induced behavioral responding in a manner distinguishable from the effects produced by either antagonist alone. Spiperone + SDZ SER 082 decreased behavioral responding across several variables in the tail pinch stressor condition and in the open field: gnawing, oral behavior directed at food, oral behavior not directed at food, line crossing, and rearing. Additionally, spiperone + SDZ SER 082 increased freezing behavior in the open field. These effects were observed when these antagonists were injected together, as well as when they were co-administered with DOI. All of these behavioral

findings are indicative of decreased motor activity. Casual observation of these animals during testing, coupled with the behavioral findings above, suggest a pharmacological, rather than physiological effect of 5-HT_2 antagonists on behavioral responding.

Antagonism of Head and Body Shakes. In addition to altering the behavioral variables discussed in the previous section, injection of spiperone + SDZ SER 082 was shown to abolish DOI-induced head shakes and body shakes. This finding is consistent with previous reports from our laboratory and others that head and body shakes are mediated at the level of the 5-HT_2 receptor (Hawkins, et al., 2002; Marek, 2003; Mitchell, Fairhall, Fretcher, and Redfern, 2003; Koskinen, Haapalinna, and Sirvio, 2003). Combined injections of spiperone and SDZ SER 082 were equally as effective as SDZ SER 082, as they both abolished head shakes. However, the same combination of antagonists abolished body shakes, whereas individual antagonists were without effect. Thus, it would appear that simultaneous administration of 5-HT_2 antagonists was more effective than either antagonist injected alone. This finding should be interpreted cautiously, as it is possible that the pharmacological effects of multiple antagonists outweigh their apparent antagonistic effects.

Hypothesis 6: Subcutaneous Injection of DOI Will Have an Anxiolytic Effect on Animal Behavior in the Open Field

DOI consistently reduced rearing, while increasing the number of head shakes, in the open field. DOI also increased body shakes, relative to saline, in experiment 5.

Rearing. DOI's effects on reducing rearing can be interpreted alternatively as a decrease in exploratory behavior (anxiogenesis), a decrease in escape behavior (anxiolysis), or general motor impairment. It seems unlikely that this effect on rearing is due to motor impairment, as line crossing, freezing, and rotarod performance were unaffected by DOI.

Thus, the third interpretation will not be addressed in the current discussion. A preponderance of rodent studies classify reductions in rearing as an indication of anxiogenesis (To and Bagdy, 1999; Rogers, et al., 2000; Singh, Jaiswal, Singh, and Bhattacharya, 1998; Osborn, Yu, Gabriel, and Weinberg, 1998; Broderick, Hope, and Jeannot, 1998; Cornwell-Jones, et al., 1992). In rare cases, reductions in "vigilant" rearing, whereby an animal rears in response to a predator's odor, have been reported as an anxiolytic effect (Dielenberg and McGregor, 2001). Given the lack of evidence that DOI induces motor impairment, it is concluded that DOI alters rearing in a manner consistent with an anxiogenic behavioral profile. This finding stands in contrast to the hypothesis that DOI would reduce stress responding to tail pinch, a discrepancy that may be accounted for by the Deakin and Graeff theory of 5-HT and anxiety.

Dual-activation Theory of 5-HT. The Deakin and Graeff theory of 5-HT and anxiety proposes that two serotonergic pathways selectively mediate distinct physiological responses to distal and proximal threats. The former pathway is composed of the dorsal raphe nuclei, which innervate areas of the amygdala and frontal cortex. Upon activation, this 5-HT pathway is thought to produce anxiogenic behavior in response to distal threats. The latter 5-HT pathway, composed of the dorsal raphe and its connections to the periventricular and periaqueductal gray matter, is thought to produce an anxiolytic response to unconditioned proximal threats. Although the current study was not explicitly designed to distinguish between proximal and distal threats, it is reasonable to conclude that tail pinch stress may be classified as a stimulus that produces unconditioned fear by way of proximal threat. Similar parallels can be drawn between the open field stressor and the distal threat of the Deakin and Graeff theory, inasmuch as this model represents an unfamiliar, anxiety-provoking

environment that is not characterized by an immediate physical threat to the animals involved.

The findings of the current study are consistent with the dual-activation theory in that DOI was shown to reduce stress responding to a proximal threat (tail pinch) and increase anxiogenic behavior in the open field. However, further examination of selective serotonergic compounds in experimental models designed to accurately represent the constructs of proximal and distal threat is warranted.

Head and Body Shakes. DOI consistently elevated the frequency of head and body shakes when administered in medium and high doses. This effect has been observed in our laboratory and others (Hawkins, et al., 2002; Marek, 2003; Mitchell, Fairhall, Fretcher, and Redfern, 2003; Koskinen, Haapalinna, and Sirvio, 2003; Whitton, Sarna, Datla, and Curzon; Wettstein, host, and Hitchcock, 1999) and is generally regarded as an effect of 5-HT_2 agonists unrelated to stress.

Hypothesis 7: The Effect of DOI on Open Field Behavior Will be Reduced by Spiperone, SDZ SER 082, or a Combination of the Two Antagonists

Experiments 4 and 5 examined the effects of 5-HT_2 antagonists on DOI-induced behavior.

Single Antagonist (Experiment 4). Others have concluded that DOI's effect on inducing head shakes is due to activation of the 5-HT_{2A} receptor (Mitchell, et al., 2003; Islam, Thompson, Akhtar, and Handley, 2004; Marek, 2003; Izumi, et al., 2002; Kulikov, et al., 1997, Gauffre, Aguerre, Mormede, and Chaouloff, 1997, Dursun and Handley, 1996). Additional support for the role of the 5-HT_{2A} receptor in mediating head shakes has come from studies of genetically-altered rats. Central glucocorticoid receptor "knockdown" rats, altered to express only 20% of normal glucocorticoid receptors, demonstrate elevated levels

of 5-HT_{2A} protein mRNA in the hypothalamus and frontal cortex, as well as hypersensitivity to DOI-induced head shakes (Islam, et al., 2004). The implications of this study are twofold: 5-HT_{2A} receptors are largely responsible for mediating head shakes, and that glucocorticoid receptors may regulate 5-HT_{2A} receptor expression. In Experiment 4 of the present study, however, the 5-HT_{2A} antagonist spiperone did not block DOI-induced head shaking, while the 5-HT_{2C} antagonist SDZ SER 082 completely abolished it. As such, the current study indicates that the 5-HT_{2C} receptor may also play a role in mediating head shaking in rodents.

Multiple Antagonists (Experiment 5). Combined injections of spiperone + SDZ SER 082 were shown to abolish DOI-induced head shakes and body shakes in Experiment 5. This finding is consistent with reports that ketanserin, a 5-$HT_{2A/2C}$ antagonist, reverses DOI-induced head and body shakes (Dursun and Handley, 1996; Fone, Robinson, and Marsden, 1991) Administration of spiperone + SDZ SER 082, as well as DOI + spiperone + SDZ SER 082 also resulted in decreased line crossing and rearing and increased freezing time in the open field. Informal observation of these animals, coupled with analyses of the aforementioned behaviors suggests a pharmacological effect of multiple antagonists, rather than a specific ability of these compounds to mediate stress-related behavior.

Hypothesis 8: The Administration of DOI, Spiperone, SDZ SER 082 and Combinations Thereof, Will Not Impair General Motor Function, as Measured by Rotarod Performance and Ambulation in the Open Field

No combination of drugs was found to alter rotarod performance significantly. However, co-administration of SDZ SER 082 and spiperone, as well as the co-administration of DOI, spiperone, and SDZ SER 082 seemed to produce motoric impairment. Although the statistical analysis of rotarod data from these groups was not significant, there was a trend toward reduced rotarod time for animals in each of the aforementioned drug conditions. In

Experiment 5, the respective means for saline, OI 0.5, spiperone + SDZ SER 082, and DOI + spiperone + SDZ SER 082 were as follows: 29, 29, 20, and 17 of a possible 30 seconds. As mentioned previously, any deficits in rotarod performance were rare in these experiments. The largest deviation from the rotarod time criterion in Experiments 1-4 was approximately 5%, contrasted with the 34% and 44% deficits in total rotarod time observed after administration of spiperone + SDZ SER 082 and DOI + spiperone + SDZ SER 082 in Experiment 5.

In the open field also, the co-administration of SDZ SER 082 and spiperone, as well as the co-administration of DOI, spiperone, and SDZ SER 082, seemed to impair motor activity, as indicated by increased freeze time and decreases in rearing and line crossing. These behavioral effects are suggestive of overdose.

Conclusion

The most consistent findings of the current study are that DOI attenuates rearing and oral behavior directed at food, while increasing the frequency of head and body shakes in the open field. DOI's dose-dependent effects on tail pinch-induced oral behavior indicate that this compound has the potential to reduce responding to proximal threats. Conversely, DOI's effects in the open field suggest an anxiogenic response to a novel, threatening environment. The observation that DOI produced opposing effects in separate behavioral models may be accounted for by the 5-HT dual-activation theory, discussed previously (Graeff, et al., 1996).

Administration of spiperone, SDZ SER 082, or a combination of the two drugs, did not block the effects of DOI on oral behavior or rearing. While DOI-induced head shaking was antagonized by co-administration of the 5-HT_{2C} antagonist, SDZ SER 082, as well as by a combination of SDZ SER 082 and spiperone, it is concluded that neither antagonist was able to alter stress-related behavior induced by the administration of DOI.

Future research should be directed at elucidating the brain areas or peripheral structures that mediate relevant stress-induced behavior. It may be necessary to selectively target brain areas of interest (such as the PVN), as blood-brain permeability remains a potential confound in studies involving peripheral injection of drugs. Additionally, the development of agonists selective for the various 5-HT_2 receptor subtypes which readily cross the BBB may prove beneficial in related behavioral research.

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Vita

John Hearn is a native of Ruston, Louisiana. He received a Bachelor of Science degree in psychology from Louisiana State University in 2002. Currently, he is a graduate student in the doctoral program of the Department of Psychology at Louisiana State University, and will earn his master's degree in May, 2005.